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SUBDIVISION G

PRODUCT PERFORMANCE

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As a guideline for the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), Subdivision G provides guidance to the registrant on developing product performance data that the Agency may require to demonstrate the effectiveness of their presticide product in controlling the pests specified in the claims on their product label. This subdivision is organized into section series which describe efficacy data for specific types of pesticides used on distinct classes of pests. Guidance on test methodology and protocols to support label claims are also presented. Discussions concerning when the submission of efficacy data is required are also presented as a foundation for the regulatory pesticide data requirements (40 CFR Part 158). Submission of efficacy data is generally waived, except for products claiming control of pest microorganisms that pose a threat to human health and whose presence cannot be observed by the user, including microorganisms infectious to man in the inanimate environment and in situations where the Agency may require data on a case-by-case basis.

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DISCUSSION ON
SUBDIVISION G PRODUCT PERFORMANCE
TABLE OF CONTENTS

FOREWORD vi

I. PHILOSOPHY AND GENERAL POLICIES AFFECTING SUBDIVISION G 1

II. SCOPE AND ORGANIZATION OF SUBDIVISION G 5

III. GENERAL ISSUES 9

IV. INDIVIDUAL TEST ISSUES 15

V. USDA COMMENTS AND EPA RESPONSES 23

VI. FIFRA SCIENTIFIC ADVISORY PANEL COMMENTS AND EPA RESPONSES 28

SUBDIVISION G PRODUCT PERFORMANCE
TABLE OF CONTENTS

Section

OVERVIEW, DEFINITIONS, AND GENERAL CONSIDERATIONS

90-1 Overview: product performance 32
90-2 Definitions 36
90-3 General considerations 37
90-30 Acceptable methods 49

EFFICACY OF ANTIMICROBIAL AGENTS

Subseries 91A: PUBLIC HEALTH USES 50

91-1 General requirements 50
91-2 Products for use on hard surfaces 52
91-3 Products requiring confirmatory data 57
91-4 Products for use on fabrics and textiles 59
91-5 Air sanitizers 63
91-6 Products for processing and industrial uses 65
91-7 Products for control of microbial pests associated with human and animal wastes 65
91-8 Products for treating water systems 66
91-30 Acceptable methods 70

Subseries 91B: NON-PUBLIC HEALTH USES 84

91-51 General considerations 84
91-52 Products for use on hard surfaces 86
91-53 Products for use on fabrics and textiles 87
91-54 Products for processing and industrial uses 89
91-55 Products for control of microbial pests associated with human and animal wastes 93
91-56 Products for treating water systems 94
91-57 Antimicrobial agents sold only for formulation use 96

EFFICACY OF AQUATIC PEST CONTROL AGENTS

92-1 General considerations 97
92-2 Aquatic herbicides 98
92-3 Swimming pool algicides 104
92-4 Industrial cooling water microbicides 105
92-5 Pulp and papermill water systems microbicides 108
92-6 Secondary oil recovery systems microbicides 109
92-7 Antifouling biocides 109
92-10 Footnotes to Series 92 Sections 111
92-20 Acceptable methods 112

EFFICACY OF FUNGICIDES AND NEMATICIDES

93-1 General considerations 130
93-2 Definitions 130
93-3 Performance standards: acceptable levels of pest control 130
93-4 Products for use against above-ground plant pests 131
93-5 Products for use against soil-borne plant pests 132
93-6 Products for post harvest use on fruits and vegetables 133
93-7 Products for use as grain preservatives 134
93-8 Products for use as seed treatments 135
93-9 Products for use on ornamental and flowering plants 136
93-10 Products for use on bulbs, corns, and tubers 136
93-11 Products for use on trees 137
93-12 Products for use on turf 137
93-13 Treatments for wood and wood products 138
93-14 Treatments for industrial materials and equipment 139
93-15 Products for control of mold and mildew on surfaces 140
93-16 Products for control of organisms producing mycotoxins (Reserved) 140
93-30 Acceptable methods 141

EFFICACY OF TERRESTRIAL HERBICIDES, PLANT REGULATORS, DESICCANTS, AND DEFOLIANTS

94-1 Overview 166
94-2 General considerations 166
94-3 Terrestrial herbicides 173
94-4 Plant regulators 178
94-5 Desiccants 189
94-6 Defoliants 193
94-30 Acceptable methods 196

EFFICACY OF INVERTEBRATE CONTROL AGENTS

95-1 General considerations 230
95-2 Foliar treatments 232
95-3 General soil treatments 242
95-4 Lawn and turf treatments 245
95-5 Outdoor woody ornamental plant treatments 247
95-6 Greenhouse floricultural treatments 251
95-7 Shade tree and forest land treatments 255
95-8 Livestock, poultry, fur, and wool-bearing animal treatments 262
95-9 Treatments to control pests of humans and pets 262
95-10 Mosquito, black fly, nonbiting midge, and biting midge (sand fly) treatments 264
95-11 Premises treatments 267
95-12 Structural treatments 271
95-13 Stored product treatments 274
95-14 Fabric treatments 276
95-30 Acceptable methods 279

EFFICACY OF VERTEBRATE CONTROL AGENTS

96-1 General considerations 287
96-2 Fish control agents 289
96-3 Aquatic amphibian control agents 291
96-4 Terrestrial amphibian and reptilian control agents 293
96-5 Avian toxicants 295
96-6 Avian repellents 297
96-7 Avian frightening agents 300
96-8 Mole toxicants 303
96-9 Bat toxicants and repellents 306
96-10 Commensal rodenticides 307
96-11 Rodenticides in orchards 310
96-12 Rodenticides on farm and rangelands 313
96-13 Rodent fumigants 316
96-14 Rodent repellents on tree seeds 318
96-15 Rodent repellents on cables 320
96-16 Rodent reproductive inhibitors 323
96-17 Mammalian predacides 327
96-18 Domestic dog and cat repellents 329
96-19 Browsing animal repellents 333
96-25 References to §§ 96-2 thru -19 337
96-30 Methods and protocols 340

FOREWORD

As a guideline under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), Subdivision G provides guidance to registrants on developing product performance data that the Agency may require to demonstrate the effectiveness of their pesticide product in controlling the pests specified in the claims on their product label. Submission of efficacy data is generally waived, except for products claiming control of pest microorganisms that pose a threat to human health and whose presence cannot be observed by the user, including microorganisms infectious to man in the inanimate environment and in situations where the Agency may require data on a case-by-case basis. The waiver of efficacy submission does not relieve registrants of their responsibility for marketing products effective for their proposed claims. Under certain situations when the Agency will request submission of efficacy data, this subdivision provides the registrant with guidance, procedures, and test protocols useful for evaluating product performance.

Subdivision G is a nonregulatory companion to 40 CFR Part 158, Data Requirements for Registration. Public comment on Subdivision has been taken in a series of public meetings, the last of which was held in July, 1982. Data requirements established by 40 CFR Part 158 are discussed in Subdivision G so that it can be read as a complete package and so that product performance testing procedures can be explained in their proper context.

I. PHILOSOPHY AND GENERAL POLICIES AFFECTING SUBDIVISION G

A. Philosophy

This subdivision of the pesticide registration guidelines concerns the effectiveness of pesticide products. Data developed under this subdivision are designed to assure that the pesticide product will control the pests listed on the label and that unnecessary pesticide exposure to the environment will not occur as a result of the use of ineffective products. Specific performance standards are used to validate the efficacy data in the public health related areas and include disinfectant uses to control microorganisms infectious to man in any area of the inanimate environment.

Use of these guidelines on product performance by registration applicants, coupled with Agency evaluation of the consequent data submittals serve two major purposes: protection of the environment and assurance of consumer benefit through public health protection to purchasers and users of pesticide products. Environmental protection would be achieved by making certain that applications of pesticides are fully and adequately effective for their intended purposes, so that:

(1) Undue pollution of the environment (and the consequent environmental exposure) does not result from the use of:

  (a) Ineffective products or ineffective active ingredients;

  (b) Excessive or insufficient amounts and rates of pesticides to achieve the desire effects;

  (c) Excessive or insufficient frequency of pesticide applications;

  (d) Inappropriate timing of applications, as, for example too early or too late, or out of season;

  (e) Impractical product mixtures containing certain active ingredients, which are rarely, if ever, needed under most pest control circumstances; and

  (f) Unnecessary use in areas or situations, or on sites where pest control is not needed.

(2) The user's (applicator's) time, labor, equipment, and energy needed to achieve the desired effect are most efficiently used; this concurrently constitutes a major benefit toward improved safety for both humans and the environment, since reduced opportunity then exists for accidents, needless exposures, bioaccumulation, increased animal tolerance to pesticides, and other similar hazards.

In situations where efficacy data is still required to be submitted, consumers benefit because the use of proper testing procedures will help insure that label claims are meaningful and truthful, and that the product label instructions are relevant and practical for safe and effective use of each product.

B. Waiver of Data Requirements Pertaining to Efficacy.

A detailed discussion of the expansion of the efficacy data waiver appears in the preamble to the proposed Section 3 revisions as Part 162 Subpart A - Registration, Reregistration and Classification Procedures (40 CFR Part 162). This discussion is quoted below in its entirety:

"As previously discussed, in 1979 the Agency issued regulations implementing serveral provision of the 1978 FIFRA amendment (44 FR 27933, 40 CFR, Part 162; May 11, 1979). Among the provisions implemented was the efficacy data waiver authority provided by Sec.3 (c)(5) of FIFRA. The Agency defined in §162.18-2(d) the circumstances when efficacy data were required to be submitted as a matter of course. Other requirements that efficacy data be submitted were generally waived.

"The Agency proposed in the Regulations for Registration, Reregistration and Classification Procedures (47 FR 40659, 40 CFR, Part 162, September 15, 1982) to extend the efficacy data waiver to additional use patterns, and it will include all registration actions, both conditional and unconditional. As stated in its previous waiver, the Agency's primary mandate under FIFRA is to evaluate the health and safety aspects of pesticides. Experience under the previous waiver policy indicates that there have been few complaints to the Agency of nonefficacious products being marketed, and the Agency is confident that its efficacy data waiver has occasioned little, if any, serious user dissatisfaction.

"The Section 3 Regulation §162.18-2(d)(2) concerning efficacy data is proposed to be revised as follows:

"(1) Efficacy data. (i) Efficacy data, in accordance with Subdivision G of the Registration Guidelines, for each product that bears a claim to control pest microorganisms (except bacteria, pathogenic fungi, or viruses living on or in other animals) that pose a threat to human health and whose presence cannot readily be observed by the user, including, but not limited to, microorganisms infectious to man in any area of the inanimate environment.

"(ii) Efficacy data, in accordance with Subdivision G of the Registration Guidelines, for each product for which a new or added use is proposed, if the product contains an active ingredient some use of which has been suspended, cancelled, or is the subject of a Notice issued under Section Series 162.11(a)(3)(ii) and the risks, identified in the Notice or suspension/cancellation action, may reasonably be anticipated as a result of the new use.

"(iii) Efficacy data requested by the Agency for any product, registered or proposed for registration, when:

"(A) A lack of efficacy has been reported for it;

"(B) The Agency needs such data to evaluate benefits of the pesticide (or of alternative pesticides) when substantial risks have been identified; or

"(C) Factors exist that make submission of such data necessary or desirable to support the presumption that it is efficacious.

"(iv) Products which are inefficacious may violate FIFRA section 3(c)(5)(A). Applications to register products which do not meet the requirements of the Act will be denied; registrations of products themselves may be deemed misbranded per FIFRA section 2(q)(1)(A) and section 12(a)(1)(E) or (F).

"Those products for which an efficacy data requirement was continued in 1979 were products which, if they lacked efficacy, could potentially have significant public health effects, such as mosquito control products, rodenticides, certain other invertebrate and vertebrate control agents, and antimicrobial products. The Agency now believes that because many of the "public health" use patterns identified at that time are more of an aesthetic and nuisance problem than one of public health and are adequately covered, in any case, by other regulatory mechanisms offering assurance that the products are efficacious, and because the efficacy of products for other of these uses is adequately discernible by the user, marketing of inefficacious products is unlikely. The public health authorities of states and localities, for example, have the expertise to determine the efficacy of a product used for rodent control. Mosquito control districts offer similar expertise with respect to mosquito control products.

"Several State pesticide regulatory agencies continue to require efficacy data to evaluate the pesticide under conditions of use within their States. The State Cooperative Extension Services use such data in making recommendations to growers within the State. Efficacy data are particularly important to the State in administering registrations for special local needs under FIFRA Sec. 24(c), and in determining suitable pesticides for use under the emergency exemption provisions of FIFRA Sec. 18.

"The Agency is proposing to extend its current waiver to efficacy data for all uses of pesticides except those where control cannot reasonably be observed or determined by the user and lack of control results in a clear adverse health effect. Efficacy data would continue to be required for products bearing claims for control of pest microorganisms that pose a threat to human health and whose presence cannot be readily observed by the user, including, but not limited to, microorganisms infectious to man in any area of the inaminate environment, and the products claiming control of mycotoxin-productng fungi. All other efficacy data requirements would normally be waived. The specific uses that require efficacy data are specified in Part 158 - Data Requirements for Registration in §158.32 with references to the testing methodology and protocols in Subdivision G - Product Performance. The level of label claims is described in Subdivision H - Labeling Guidelines for Pesticide Use Directions (§§ 100-106) and guidance is also given to test methodology needed to support these claims when necessary.

"The Agency expect and believes that registrants will ensure that their products are efficacious when used in accordance with label directions and commonly-accepted pest control practices. Under the statute, the registrant still has the responsibility to ensure a product satisfies its label claims. The Agency would take corrective action on a product including, when necessary, enforcement or cancellation actions, since the registrants must still comply with the law. In addition, pesticide producers are aware that they are potentially subject to damage suits by the user community if their products prove ineffective in actual use. Such litigation can be damaging to the company's reputation and future sales. It is in a company's own best interest to continue high quality efficacy data development and to market only products demonstrated to be effective.

In cases where efficacy data review is still required, the Agency determines that: label claims for control of pests or obtaining specific plant/ animal responses are verified by scientific evidence; label directions for use are consistent with commonly recognized practices of pesticide use; and label directions for use are supported by scientific evidence based on testing of the pesticide under the most suitable conditions for predicting product performance under the variety of use conditions likely to be encountered.

"Under this proposal, the Agency retains the right to require the submission of efficacy test data or other evidence, on a case-by-case basis, for any pesticide product registered or proposed for registration, for which a lack of efficacy has been reported, for evaluation of product benefits when product risks are substantial, or when other factors exist which make submission of such data necessary or desirable to support the presumption that it is efficacious. If there is evidence (such as a significant rise in complaints from user groups, scientific societies, trade associations, or the general public) to establish that this regulatory relief policy is being abused, the Agency would reconsider its waiver policy. The Agency is building links to various organizations that are knowledegeable of efficacy matters through a product performance information network. Also, the Agency is actively pursuing the establishment of formal relations with various departments, such as the U.S. Department of Interior's Fish and Wildlife Laboratory in Denver to conduct rodenticide surveillance and with professional organizations such as the National Pest Control Association and American Hospital Association to aid in efficacy evaluation when the surveillance network or other sources indicates the need."

II. SCOPE AND ORGANIZATION OF SUBDIVISION G

A. Scope

The data generated by the product performance studies described in these guidelines are used by the Agency, when applicable, along with other data, to assess the efficacy of pesticide products as part of making the determination as to whether EPA should register the product. The guidelines also represent the procedures found to be useful in the Agency's review of product performances when efficacy data submission is requested on a case-by-case basis.

These proposed guidelines specify:

(1) The conditions under which each particular data requirement is applicable to a pesticide product;

(2) The performance standards for acceptable testing, stated with as much specificity as the current scientific disciplines can provide; and

(3) The information to be included in a test report.

The guidelines also indicate when applicants should consult with the Agency before initiating certain tests, and when the submission of efficacy data is waived. In addition, each section series provides useful examples of acceptable protocols for conducting product performance testing.

B. Section Series.

The Agency has greatly expanded the areas covered in these guidelines since the 1975 proposal (40 FR 26802 June 25, 1975). This subdivision is organized into seven series of sections, one covering overview, definitions, general considerations, and the other six series of sections covering guidance on efficacy data for specific types of pesticides used on distinct classes of pests. These sections contain guidelines and information needed by the Agency to evaluate efficacy and establish limitations for specific use patterns. Subdivisions G and H (Labeling Requirements for Pesticides and Devices) have parallel organization to aid in locating label claims, and supporting performance standards and test methodology when applicable.

Basic considerations which apply to all sections except the antimicrobial series (§§ 91-1 through -9) appear in the Overview (§ 90-1) and General considerations (§90-3). Included in the Overview and General considerations sections are the efficacy data waiver policy, definitions, general considerations on test standards, plot sizes, geographical distribution, application methods, dosage rates, experimental design, adverse effects, assembly of these reports, and related information for submittal to the Agency.

Sections 91-1 through -8, Efficacy of Antimicrobial Agents, include data requirements on sterilizers, disinfectants, virucides, certain fungicides (those to control fungi pathogenic to man or domestic animals), sanitizers, and bacteriostats. These section are concerned with public health related uses only. Section 91-30 provides recommended methods for satisfying data requirements as well as supplemental recommendations for expanding methods for specific claims and use patterns. Subseries 91B are concerned with providing guidance on non-public health uses only. Among the target pests which are covered in these section series are those posing a potential health hazard to man or animals, or the ones causing spoilage, deterioration, or the production of offensive odors in substrates in which they grow.

Sections 92-1 through -7, Efficacy of Aquatic Pest Control Agents, includes testing and performance guidance on pesticides used in aquatic environments, such as aquatic herbicides, swimming pool algicides, industrial cooling water microbicides, pulp and papermill water system microbicides, secondary oil recovery system microbicides and antifouling paints. Section 92-30 lists acceptable test methodologies and references which serve as a guide for developing suitable test methods. This section series concerns efficacy data for use pattern of pesticides all of which are routinely waived for submittal.

Section 93-1 through -16, Efficacy of Fungicides and Nematicides, includes testing and peformance guidance for fungicides and nematicides used to control above-ground plant pests and soilborne pests; disease and deterioration organisms on post-harvest fruits and vegetables, grains, seeds, ornamental plants (including bulbs, flowers, and trees), turf areas, wood and wood products (to prevent rot); industrial materials and equipment (to prevent deterioration, mold, and staining by fungi to products such as fabrics); and mold and mildew on surfaces. Section 93-30 contains acceptable test methods for evaluating control of certain fungal pests on inanimate surfaces, polymeric materials, and plant parasitic nematodes. This section series provides guidance for developing data on non-public health uses of pesticides, all of which are routinely waived for submission except for products claiming control of mycotoxin-producing fungi.

Section 94-1 through -6, Efficacy of Terrestrial Herbicides, Plant Regulators, Desiccants and Defoliants, includes guidance on these types of pesticides. The section on plant regulators provides data guidance on products claiming increased yields, flowering, fruitset, berry size of seedless grapes, and products enhancing abscission, inhibiting tobacco sucker growth, inhibiting apple scald, etc. Guidance for herbicides, desiccants, and defoliants are also arranged by crop and use. Section 94-30 contains references to herbicide evaluation and to specific conditions for evaluation of each type of plant regulator discussed in the guidelines. This section series contains efficacy data for use patterns of pesticides, all of which are routinely waived for submission.

Sections 95-1 through -14, Efficacy of Invertebrate Control Agents, includes data guidance for invertebrate pesticides which are substances or mixtures of substances intended for preventing, destroying, or repelling invertebrate animals declared to be pests, including any member of the class Insecta, and other allied classes in phyla Arthropoda and Mollusca (including but not limited to spiders, slugs, sowbugs, centipedes, and snails), but excluding the class Nematoda (nematodes) of the phylum Nemathelminthes. These target pests are present at a variety of sites, including households, building premises, agricultural and ornamental crops, in or on livestock, on humans and pets, wood structures, and in stored products. Section 95-30 contains references to acceptable test protocols. This section series concerns data for use patterns of pesticides, for which product performance data are routinely waived for submission.

Section 96-1 through -19, Efficacy of Vertebrate Control Agents includes data guidance for vertebrate pesticides which are substances or mixtures of substances intended for preventing, destroying, repelling, or mitigating any vertebrates including fish, amphibians, reptiles, birds, and any wild and domestic mammals (except man). Vertebrate pesticides include oral, dermal, and inhalation toxicants; irritants; repellents (odor, taste, or tactile reaction); chemical frightening agents; anaesthetizing chemicals; and reproductive inhibitors. The sections of acceptable methods are composed of two parts: one containing references on supplementary information about test procedures (§96-25), and the other containing acceptable methodologies (§96-30). This section series concerns use patterns of pesticides, for which product performance data are routinely waived for submission.

C. Organization of Sections within Subdivision G.

1. General requirements or considerations. Each section series on specific pesticides begins with a general requirements or general considerations section. This section discusses scope, definitions, general data considerations, and general performance standards pertinent to the specific types of pesticides. The section also emphasizes any information that differs from or supplements the information in Section series 90-1 through 3 on Overview, Definitions, and General Considerations.

2. Section subdivisions. Most of the Section series contain sections which cover products used on or in specific sites (e.g., hard surfaces, swimming pools, turf, crops, orchards, and industrial cooling water systems). Other section series are organized according to organism (e.g,, avian toxicants, fish control agents, mole toxicants, and soil-borne pests). Further subdivisions of these sections are bases on sites where the pests are found.

3. Suggested performance standards. Many of the individual sections contain performance standards. A performance standard represents the lowest level of product performance which would normally be acceptable for protecting the public health, when required, or for economic control of a specific sites for a given pest or pest combination. The proposed performance standards are usually expressed as percentages of pest control and serve as general guidance. Public health related label claims to be supported should meet the specific performance standards. If these standards are not met, the registrant may choose to use a lesser label claim or put a disclaimer on the label that the product did not meet EPA standards for effectiveness.

4. Effectiveness paragraphs. Each section describes the criteria used to determine the effectiveness of the product in preventing, destroying, repelling, or mitigating a pest; accelerating or retarding the rate of growth of a plant or insect or otherwise altering the behavior of the pest or host organism; or defoliating plants or artifically accelerating the drying of plant tissues. Effectiveness as determined by experiments could also be designed to obtain information on adverse effects. Adverse effects and hazards to man and the environment are discussed in Subdivisions D, E, F, J, K, L, M, and N. In addition to the adverse effects specifically evaluated by other subdivisions, this subdivision provides guidance on evaluations of other kinds of adverse effects such as deteriorated food quality, discolored and weakened fabrics, unsightly residues on plant foliage, reduced crop palatability, increase in harmful nontarget organisms, and presence of dead pest organisms as a potential food source for domestic or wild nontarget organisms.

5. Sections on Acceptable Methods. Each of the section series for specific types of pesticides contains a section on acceptable methods. Each of those sections discusses specific test methods and provides references to published literature which gives the registrant specific examples of acceptable or related test protocols. Much of the literature also aids in developing test protocols and in reporting details on evaluation of tests and experimental designs and on statistical evaluations. Several of the cited references are methods developed by consensus organizations such as those from American Society for Testing and Materials Committee E-35 on Pesticides; Association of Official Analytical Chemists; and from the Agency's Manual of Biological Testing Methods for Pesticides and Devices as an aid to the registrant.

III. GENERAL ISSUES

A. Test Standards.

This part of the discussion concerns certain issues which have arisen with respect to test standards governing methodologies. The issues discussed here concern general test standards which apply to meeting data specifications of several kinds of tests.

Issues involving technical aspects of individual tests standards are discussed in part IV of this Discussion.

1. Test substance. Final tests to support the effectiveness of a product are usually conducted with the formulation proposed for registration and frequently with the product in the same packaging intended to be used commercially. This latter test is especially important for pesticides marketed and applied directly from containers or container-devices. End-use tests using the formulated product are required in cases where formulated products found to be effective in laboratory tests are ineffective when packaged in commerical quantities, for a variety of reasons, including synergism, antagonism, physical incompatibility with inerts, short shelf-life, chemical reaction with a component added at repackaging, or improper functioning of containers which also serve as application devices. Moreover, a product initially effective at the user level may suddenly become ineffective or unusable because of a change in the can liner coating, product emulsifier, solvent, or other component used. Mixtures of two or more pesticides in a single formulation may react chemically, be physically incompatible (producing a useless product), or be mutually antagonistic or synergistic for effectiveness or adverse effects.

2. Minimum effective dose (MED) and effective dosage range (EDR). In § 90-3 General considerations, paragraph (b)(3) it is stated that the applicant demonstrate the minimum effective dose and the effective dosage range. [See the definitions in § 90-2(a) and (f).] These data are useful in risk/benefit considerations where a determination of lower dosage rates may, in some cases, allow a reduction in adverse effects or environmental contamination while still providing acceptable levels of pest control.

The need for MED and EDR would not apply to the section series 91-Efficacy of Antimicrobial Agents, for two principal reasons:

(1) For many disinfectants, the important information concerns whether or not total elimination of the target organism(s) is achieved, rather than an MED or EDR, and

(2) For many antimicrobial product applications, the extent and influence of uncontrollable factors (such as level and type of contamination, resistance of microorganisms, nature and configuration of surface, and

soil load) are too variable and great for an MED or DER to have any significant value.

(3) Plot Size. Many questions arose on the proposed guidance concerning size of the test plot or site. Several people were of the opinion that the Agency usually would request test plot sizes larger than one acre, even up to hundreds of acres, and they felt that this would invalidate most of the data developed from experiments on plots smaller than an acre. The Agency feels that small plots, properly relicated and well designed, provide very useful and valuable data. The Agency encourages small plot testing since considerably less of the pesticide would be placed in the environment than if testing were performed on larger plots. In addition, larger plots may occasionally increase the potential for hazard to wildlife.

B. Effectiveness Evaluations.

1. Suggested performance standards. The product performance guidelines suggest specific performance standards for several different areas. These standards would benefit the Agency and the consumer because they would ensure that the products are useful and will control the pests indicated on their labeling. The performance standards have been discussed extensively at the Agency and are considered to represent suitable guidances at this time. Many of the performance standards have been routinely used by industry and the Agency for many years. In some instances, experts throughout the country were contacted to develop some of the performance standards, as well as the Agency's own scientists at headquarters and at laboratories and field stations.

Some of the suggested performance standards are based on comparisons of the effectiveness of products to the effectiveness of standard reference chemicals. Often the performance standards are very explicit as to whether the comparison involves the amount of chemical per acre, reduction in pest levels, different target pest, or adverse effect to the crop. Some people have commented that a performance standard based on percent reduction of pests is appropriate but are concerned that applicants at times would be encouraged to use increased number of target pests in the test area to misrepresent the derived benefit.

The performance standards are useful for guidance purposes and would be applied flexibly. EPA recognizes that the level of control derived from a single pesticide dosage varies with each pest and site combination and a number of other factors, including the user group; the geographic region; crop grading and quarantine standards; users of the treated commodity; the anticipated level of pest population to be encountered by users; climatic conditions; soil textures; crop cultivars; and, in some instances, comparisons with existing control measures.

Comments already received have questioned the general performance standards in Section series 93- Efficacy of Fungicides and Nematicides, and 94- Efficacy of Terrestrial Herbicides, Plant Regulators, Desiccants, and Defoliants. Both of these section series suggest standards of 70% pest control. The performance standard is useful primarily to distinguish between products having barely adequate effectiveness and those having fully adequate effectiveness. The Agency may use this distinction to determine the appropriateness of acceptable label claims. Accordingly, when a product's performance is somewhat below the proposed performance standard, the registrant could label his product with a lesser degree of control claims, such as "aids in control," "partially controls," or "suppressses," as opposed to "control" or kills claims.

In certain cases, less than 70% control may be acceptable, for example, when the target pest is particularly resistant or when no suitable alternative or more effective pesticide exists. Such cases would need supporting background information, when applicable.

A suggested general performance standard of 70% control is not adequate for some pest problems. For example, when human safety is involved, as in the case of many disinfectant uses, the Agency generally would require close to or not less than 100% control. Comments received from USDA recommended general 70% performance standards, but presumably these were recommended only in relation to pesticides for use in crops. Actually, 70% control for pests of humans and pets would not be totally unacceptable from a nuisance control standpoint, but it would provide essentially no protection of health to humans or domestic animals. To support the Agency's mandate to protect health and through persuasion of arguments submitted by the Chemical Specialties Manufacturing Association (CSMA), certain pesticide performance standards were raised from 90% to 95% control to meet acceptable levels of control that could be tolerated by humans. EPA was persuaded by these comments, for example, that 90% control of some populations of mosquitoes would not be high enough, since the uncontrolled mosquito biting rate of 50 per minute would be reduced only to 5 bites per minute, a level which would still be unacceptable to the consumer.

Also, in section series 96- on Vertebrate Control Agents, the suggested use of a general 70% performance standard for reduction in pests was considered to be inappropriate. For example, if 70% of the woodchucks in a given area were killed each year, there would still be the same potential number the next year due to their reproductive capacity. Control of other animals such as foxes and birds presents a similar problem.

With rodenticides, a 50% reduction in population control in actual use could leave a massive population not controlled and capable of quickly replacing the animals killed. Therefore, percent reduction in pest damage is often a better rating factor to use to evaluate damage potential to orchards, crops, and homes than percentage of animals killed.

The suggested performance standards are generally based on current agricultural practices in the United States. Such standards do not presently take into account performance under any of several integrated pest management (IPM) practices. Such programs do not generally result in unacceptable levels of control, but may involve lower rates or less frequent applications of pesticides. Use of pesticides in such programs should meet the suggested performance standards when evaluated in conjunction with the nonchemical practices that are an integral part of directions for use in IPM. Evaluations would be expected to take into account those situation such as when predators further reduce pest populations below the levels attained with the pesticide used alone. Periodic revisions of these guidelines will provide opportunity for greater attention to any such programs that become commonly recognized practices.

By utilizing Subdivision H and Subdivision G guidelines, the registrant would be provided an opportunity to make his own judgements regarding claims to be made on the pesticide product label. These claims could predetermine specifically what the Agency expects as to effectiveness of his products. The suggested peformance standards would provide breakpoints to aid in the determination of what the registrant will claim in his label in relation to results of testing to meet the data requirements. Registrants can distribute portions of these guidelines to cooperators and contractors who could than more easily supply the exact information needed to support product registration.

2. Mode of action. These guidelines recommend that a description of the pesticide's mode of action be submitted or referenced if published. Commenters have expressed concern as to the amount of sophisticiation necessary for the studies to report on mode of action. Since the mode of action depends upon the individual chemical, use pattern, and type of product, the Agency decided that stating the general mode of action is adequate, e.g. that the substance acts as repellent as opposed to a nerve toxin. Although it in not intended that molecular studies on modes of action be required, the molecular mode of action, if known, should be reported.

3. Rating scales. Rating scales are often used to assess pest damage or pest control, and the guidelines often specify that such scales be used as long as each value in the scale is fully defined. Several commenters were concerned over reporting results using only the rating scales described in the draft test protocols, since many other rating scales are used in evaluating pesticide product performance. The agency feels that rating scales to assess factors such as quality, fabric damage, or algae growth, etc., would also be acceptable, provided that adequate explanation for each rating system is given. When the test specifies a certain rating system, then the specified system should be used; however, additional rating systems may also be used and results reported, if the registrant so desires.

4. Benefits data. Data submitted to the Agency should also demonstrate the benefit of the product to the user. While the Agency will not make lack of essentiality a criterion for denying registration of any pesticide, this does not mean that benefits information should not be submitted when it is required. Benefits derived from pesticides may be prevention of storage rots, decrease in disfigured fruits, yield increases, decreases in crop damage, decrease in disease incidence (e.g., Rocky Mountain spotted fever) prevention of fabric staining, prevention of lumber staining, etc. If the product is not effective and no benefit results from its use, the use of the chemical would merely add undue pollution to the environment. For further discussion of benefit data, see §90-3 General considerations.

5. Testimonials. Section 90-3 General considerations states that testimonials by experts regarding the efficacy and limitations of particular products would be acceptable as part of the data submission. Several commenters are of the opinion that the submission of testimonials would be of limited value because it is usually possible to obtain positive testimonials, and because most registrants would not be prompted to submit negative testimonial letters on their products. In addition, they commented that accepting testimonials is likely to create a burden on the Agency, since the credentials of the persons submitting the testimonials would have to be examined. The opinions of such experts may also be too biased by their position or economic relationship with the registrant. However, the Agency will determine the value of each testimonial submitted based on its merits.

C. Product Not Mentioned in the Guidelines.

The Agency has not provided protocols for the types of products which have never been submitted for registration. Accordingly, certain uses of products are not mentioned in the proposed guidelines. It should not be concluded that such products do not have to be registered, nor does it mean than there are no data requirements. Rather it only means that a number of current or unforeseen future uses of products are not covered because the methodology for testing these products has not been adequately developed. The Agency's Product Managers should be contacted directly about requirements for products not mentioned in the guidelines, since the efficacy data requirements for these products will handled on a case-by-case basis.

Also, in development of these guidelines, the Agency anticipates that, with further new product development the Agency policy and guidance may well change to reflect the new development. The guidelines will be revised to incorporate new advances in science and technology, new pesticide uses, and changes in methods development. These guidelines are meant to be dynamic, and the Agency will strive to keep current with the state of the sciences involved.

D. Separation of Agricultural Products from Household Products.

One comment received stated that the guidelines fail to recognize the difference between agricultural-commercial pesticides and household products. However, the Agency disputes this point. The guidelines are organized so that any person who is at all familiar with pesticides and their uses can distinguish between the guidance designed for agricultural products and those for household products. For example, this separation of agricultural and household products in the invertebrate area is evident in § 95-7 shade tree and forest land treatments vs. § 95-11 premises treatment.

E. Organization of Antimicrobial and Fungicide Sections.

Several commenters were concerned over the separation of nonpathogenic fungi, especially mold and mildew (§ 93-15) from the antimicrobial section series (91). They felt that all non-agricultural uses of antifungal products and algicides should fall into section series 91- Efficacy of Antimicrobial Agents. At present, all or most combination-use products have different directions for use and site of application due to the inherent differences between mold and mildew pest problems and antimicrobial pest problems. The Agency recognizes that there are numberous approaches that could be used in presenting the information contained in the guidelines, one logical approach seems to be grouping the information according to general type of pest organism, since the testing and labeling considerations primarily relate to the type of pest organism and nature of the pest problem, and only secondarily to the sites of application and other factors. Additionally, this type of approach complements the scope of expertise of scientists developing, using and evaluating the data.

The Agency would like to know what specific types of problems have occurred as a result of handling mold and mildew and antimicrobial products separately (as reflected in the guidelines). This has been the policy for some time and the Agency is not aware of any important problem which warrants changing this practice.

F. Toxicity Test Recommended for Animal Repellents.

Some commenters questioned the need for toxicity tests data on rats for animal repellent products designed to repel other vertebrate animals (but not rats). See, for example, the rat acute oral LD50 studies in §96-18 Domestic dog and cat repellents, and in §96-19 Browsing animal repellents (designed to repel deer, elk, and lagomorphs).

First, the toxicity tests on rats will give an indication of the lethal dose for the target species. The LD50 value will allow EPA to determine whether the dose level which effectively repels the target species is close to the lethal dose. If the Agency concludes that there is not an adequate margin of safety between the repellent dose and the lethal dose, further testing may be needed.

Second, the rat studies are less expensive and easier to perform for toxicological tests than studies involving larger target species. Also, the toxicity methodology for large mammals is not yet as well developed as it is for rats, and the legal problems associated with large animal studies can be avoided.

A third reason for the toxicity test is to aid in determining the mode of action of the repellents [e.g., whether it works as a poison or through tactile response (by rapid inflammation of tissue)]. An understanding of the mode-of-action would aid in developing more accurate labeling instructions and performance standards.

IV. INDIVIDUAL TEST ISSUES.

A. Simulated Use or Actual Use Studies for Antimicrobial Agents.

In section series 91 Efficacy of Antimicrobial Agents, more emphasis is placed on the use of test methods which more closely reflect actual conditions of use. This new emphasis would be particularly valuable for methods to support claims for "one-step" cleaner-disinfectants, surgical instrument sterilizers and disinfectants intended for re-use, and residual bacteriostatic claims for treated surfaces or materials. As a result of this new emphasis, the following problems would likely be alleviated:

- (1) Dependence on zone-of-inhibition methods that are not related to actual use situations;

- (2) Dependence on many standard methods such as AOAC procedures employed for disinfectant-type products, which do not adequately represent the manner in which these products are intended or claimed to be used;

- (3) Dependence on standard methods which do not address such factors as presence of organic soil, varying exposure period, or re-use of prepared solutions;

- (4) Propensity for label claims and directions on certain products not to be restricted to those which could be actually supported by the standard test methods.

B. Uniformity of Test Microorganisms, Carrier, and Batch Replication Required for Testing Antimicrobial Agents.

The test standards which have applied in the past to the performance of efficacy studies on antimicrobial products have been revised for these guidelines to provide greater uniformity and reliability in testing. In the past, the number of carriers (i.e., surfaces such as porcelain, stainless steel, and surgical suture loops on which microorganisms are deposited) required for basic efficacy testing of disinfectants varied from 30 to 60, depending on whether the organisms was considered primary or secondary for either the hospital or general level of disinfectant. This subdivision provides that all basic efficacy data for disinfectants will be conducted using 60 replicates per type of carrier per test microorganism per sample. Also, one of the three microorganisms required in the testing of general disinfectants was not specified. The guidelines now provide that only two specified test microoganisms, Staphylococcus aureus and Salmonella choleraesuis, be required for efficacy testing for general disinfectants. For hospital disinfectants, S. aureus, S. choleraesuis, and Pseudomonas aeruginosa are required, as was the case previously. Finally, a separate 60-day shelf-life stability sample of the product was originally required to be tested in addition to three different freshly-prepared batches. In the current guidelines, three samples of the product, representing 3 different batches (one of which must be at least 60 days old), would be required; this new requirement would eliminate the previous need for a separate shelf-life stability study. The current, simplified requirements would result in fewer replications being performed in the case of limited and general disinfectants, and would provide the reliability indicated in the Use dilution method and the AOAC Germicidal spray products test. More replication is required for hospital disinfectants because greater confidence is needed so that these products will achieve their important function.

The Agency has received several comments regarding the data requirements to support sterilization claims. Tests to provide the basic efficacy data for sterilizers are also to be conducted using 60 replicates per type of carrier per test organism per sample. This requirement is analogous to the requirements for disinfectants, and provides for greater reliability in assessing the effect of the two types of carriers specified in the AOAC Sporicidal test. The performance standard of no failures in 60 replicates to demonstrate efficacy of products intended as sterilizers is indicated in this AOAC method. The specific performance standard for sporicidal activity is not considered in the guidelines, since the sporicide category has been eliminated, as explained in paragraph C (below).

C. Sporicide Category of Antimicrobial Agency Eliminated.

For purposes of the guidelines, the Agency proposes to treat sporicides and sterilizers alike. It appears that most consumers consider "sporicidal" and "sterilizing" activity to be synonymous. In the past, the Agency has accepted a lower level of efficacy for sporicidal products than for sterilizers. This situation could have potentially hazardous consequences if users expected sterilization and obtained only sporicidal control. Therefore, in order to avoid the marketing of products with claims which might be misunderstood, the Agency will henceforth deem "sterilizers" and "sporicides" to be synonymous; products not killing all organisms, including spore-forming bacteria, may be deemed "disinfectants" but would not be deemed "sporicides."

D. Use of Neutralizers in Microbiological Assay Systems.

Several commenters suggested that evidence to show that the neutralizer mentioned in § 91-1(b)(3) inactivates the active ingredient(s) and does not possess antimicrobial activity per se should not be required where there are known standard neutralizers for the active ingredients in the formulation such as those documented in the literature or cited in the AOAC method.

Most of the standard neutralizers are designed for a specific type of active ingredient. Because most antimicrobial products are complex formulations containing several active ingredients, the use of a neutralizer designed for one active ingredient may not effectively neutralize the formulation. Additionally, there is evidence that standard neutralizers such as letheen broth possess antimicrobial activity against some bacteria. For these reasons, the Agency has concluded that evidence of neutralization of each antimicrobial formulation is required. Citation of existing data on the identical formulation would be acceptable in lieu of actual submission of the test data and would satisfy the intent of the requirement.

E. Status of Ingredients in Antimicrobial Products as Active or Inert.

Comments were received from the public stating that the requirement for the AOAC Phenol Coefficient method to determine the status of questionable ingredients as active or inert [see § 91-1(b)(10)] was inappropriate. The Agency agrees that more flexibility should be provided in this area and this section has therefore been revised to allow other types of testing which may be mutually agreed upon by the applicant and the Agency. It should be pointed out that it was not the intention of the Agency to require this testing routinely to determine the active or inert status of ingredients in antimicrobial pesticides, but rather to be responsive to numerous requests by applicants who want a basis for establishing that an ingredient, heretofore considered active, is actually an inert ingredient.

F. Antimicrobial Terminology

A number of comments were received from the public regarding the definitions in section series 91. The Agency did not intend to use the classical definitions for all of the terms because the definitions are designed specifically for registration activity. Also, many of these definitions are similar or identical to those set forth in the Act and at § 162.3 of the FIFRA sec. 3 regulations. Since the terms are primarily important for label claims, § 91-1(c) has been moved to Subdivision H (Labeling Guidelines for Pesticide Use Directions) at § 101-1(d).

G. Sample Requirements for Testing Products Used as Laundry Additives.

Several commenters proposed reducing the number of fabric swatches required for demonstrating disinfection. [See § 91-4(a)(1)(i)(C).] The Agency agrees with this suggestion, and has reduced the number of fabric swatches from 60 to 9. This proposal has been adopted on the basis that the amount of replication originally proposed is unnecessary where fabric swatches, rather than hard surface carries, are involved. EPA assumes that it is easier to disinfect fabric surfaces than hard surfaces. This revision should allow for considerable reduction in the amount of required replication. If subsequently submitted data does not confirm this assumption, the Agency may consider modifying the requirement to increase the number of swatches.

Comments were also received questioning the need to test both the fabric and the laundry water. Commenters felt that testing the wash water is redundant and serves no purpose, since the fabric swatches will retain some of the wash water anyway; if the organisms are not killed, the water residual in the fabric will indicate this. However, the concentration of antimicrobial content in or on the fabric may differ from that found in the water and give different results. The Agency feels that testing the wash water is necessary to show whether organisms eluted from contaminated fabric into the water can or cannot recontaminate the clothes or contaminate the clothes of the next user. This testing is required to prevent public health problems in commercial and coin-operated washing machines where cross-contamination between users may be a problem.

H. Standard Carpet Test Samples and Microorganisms.

The Agency recognizes the diversity in carpet samples and wants the carpet intended to be tested to be fully identified in the test report. Characteristics such as the pile fiber type, pile yarn weight of finished carpet, pile density, and tuft height should be included. [See §91-4(b)(1)(i).] These characteristics may affect the performance of the product. Commenters have suggested that the Agency supply specific test carpet standards, but the Agency rejects this comment until more information on suitable test standards is developed. Industry cooperation in developing suitable test carpet standards, such as through CSMA, AOAC, or ASTM, would be useful to testers, users, and evaluators of carpet sanitizers.

A commenters has suggested that *Pseudomonas aeruginosa* is a poor selection as a carpet organism because of its low recovery counts after drying. This statement is contrary to information the Agency currently has on this organism. The Agency welcomes additional test information to expand upon any problems associated with using *P. aeruginosa* as a test organism for carpet sanitizer testing, and would also welcome suggestions for any other suitable or superior organism for this test.

I. Air Sanitizer Test Methodology.

Recent public comments on test requirements in § 91-5 Air Sanitizers have suggested that there is methodology now available that provides specific test standards to evaluate air sanitizers, such as: air sampling procedures, suitable type of air filtration, suitable exhaust systems, use of *Micrococcus lysodeikticus* as a test organism, specific glycol concentrations, proper time frames for sampling, and acceptable types of air samplers. The concern by certain other commenters about the need for certain standards could be evaluated once the Agency evaluates such standards. The Agency therefore invites submittal of test protocols and related information on air sanitizer testing.

J. Use of Mixed Cultures in Testing Products for Processing and Industrial Uses.

Commenters have disputed the need for identifying each test organism as a contaminant in metal-working fluids, and for the separate testing of bacteria and fungi inocula in the test protocols. In response to these comments, the organism identification was reduced to the level of genus only. And for mixed cultures, where there is evidence that both bacteria and fungi are normally present and necessary to (re)produce the spoilage problem [as in fuels, §91-6(d)], a mixed inoculum is appropriate and should be used; where mixed cultures are not normally the case [as in metal-working fluids, § 91-6(c)], mixed cultures should not be used and the organisms should be tested separately.

Bacterial and fungal mixed cultures are special cases. When the applicant can justify the use of mixed bacteria/fungal inoculum as necessary to (re)produce a specific spoilage problem, then the use of such mixed cultures can be considered on a case-by-case basis.

A list of representative organisms in metal-working fluids has been developed by the American Society for Testing and Materials (ASTM) E35-15 Subcommittee on Antibacterial and Antiviral Agents and may prove to be useful.

K. Water Purifier Units and Bacteriostatic Water Treatement Units.

Section 91-8(a)(3) would delineate tests standards and requirements for water treatment units. The current Agency policy with respect to regulation of water treatment units was first explained in the Interim Requirements for Registration of Bacteriostatic Water Treatment Units for Home Use, published in 41 FR 32778, August 5, 1976, and in an unpublished EPA document entitled "Interim Standards for Water Purifiers." The scientific premises upon which these test procedures were based have been reexamined in view of these units and expected product performance under actual use conditions.

- 1. The "Interim Standards for Water Purifiers," which was intentionally directed solely to the pesticidal use of silver, now has limited significance because data submitted more recently by registration applicants showed that silver will not purify water at the maximum allowable concentration 50 pbb in effluent water.

- 2. The policy explained in the "Interim Requirements for Registration of Bacteriostatic Water Treatment Units for Home Use" was based on the assumption that extensive growth of saprophytic bacteria trapped within the filtering medium during stagnation periods could pose a potential bacteriological problem of public health significance. Scientific evidence published since that time has not documented or substantiated this assumption. Data developed as a result of issuance of this "Interim Requirement Notice" have failed to demonstrate either a bacteriological problem or a valid product function other than an assumed aesthetic value. Under the present efficacy data waiver policy (see Part I.B. of this discussion), effectiveness data are being waived for bacteriostatic water treatment units since only an assumed aesthetic value can be associated with these products.

If, however, scientific information indicates that a potential health hazard exists because bacteria-infested water filter units are used to process potable water, the following must be taken into consideration before an adequate testing program can be undertaken:

- (1) The level of saprophytic bacteria that would be considered to be of public health significance in potable drinking water must be defined.
- (2) The level of antimicrobial activity required to eliminate a potential health hazard would have to be at least at the sanitizing level; the inhibitory level of activity (bacteriostatis) would not be adequate.

M. Antimicrobial Agents Intended for Treatment of Municipal Drinking Water.

The Agency, under FIFRA, does not exert primary regulatory control over antimicrobial chemicals used to treat municipal water supplies for the purpose of rendering them microbiologically potable. The use of such products is regulated by EPA, in cooperation with local jurisdictions, under the Safe Drinking Water Act (PL 93-523). For example, chlorine is commonly used to disinfect water supplies, but no tolerance (under the Federal Food, Drug, and Cosmestic Act) has been granted or approved for chlorine as a pesticide in this pattern of use. The organizations within the Agency responsible for the pesticide and the drinking water programs will coordinate their efforts to provide safe and efficacious treatment of municipal drinking water. Antimicrobial agents intended for treatment of municipal water supplies will be registered for such use if they are certified and approved by the EPA Office of Drinking Water.

N. Antimicrobial Agents Sold Only as Manufacturing-use Products.

In § 91-9, the Agency formally provided requirements for efficacy testing of manufacturing-use products intended for incorporation into antimicrobial formulations. The purpose of these requirements would be to ascertain whether such products have any intrinsic value as antimicrobial agents. Comments already received have questioned the usefulness of such testing, especially in view of the efficacy waiver policy [see § 90-1(a)] which requires data submittal only for pesticides intended for public health uses. Manufacturing-use products obviously do not have a direct impact on public health, since they are not sold as such for an end use; they are, however, used in making pesticide formulations whose uses are designed to protect public health. Since testing data are already required for such formulated products intended for public health end uses, the Agency agrees that submission of efficacy data for manufacturing-use products is not necessary under the efficacy waiver policy.

O. Test Standards for Insecticide Premises Treatments.

The test standards for insecticides to be used in houses and other similar premises (see § 95-11) are based on comparison of the test substance with either the official test aerosol (OTA) or the official test insesticide (OTI). Submitted comments have questioned these test standard. First, the OTA standard contains freons which are no longer acceptable for use in pesticide products, and consequently is an inappropriate reference standard. The OTI standard, which was changed from DDT to a pyrethrums mixture, can be very difficult to adjust for dosage mortaility, and this is not very practical. Also, results in actual premises tests vs. laboratory tests often show significant discrepancies for new compounds.

Many universities have rapidly-expanding programs in urban entomology where adequate methodology is being developed. However, the procedures on premises treatements included in these guidelines are still widely recognized and provide guidance until researchers develop more accurate test methods. The Agency invites the public to submit information on new methodology in this area, and on the OTA and OTI standards and potential substitutes.

P. Field Tests vs. Lab Tests for Structural Treatments.

Section 95-12 Structural Treatments would apply to invertebrate control pesticides used in controlling pests such as wood-destroying beetles, carpenter ants, and termites. This section would need data derived from field testing. Comments already received have recommended that EPA accept laboratorys efficacy studies for purposes of evaluating the performance of pesticides intended for control of wood-destroying insects. At the present time, however, the Agency feels that the field tests for structural treatments would give the best estimate of efficacy, since laboratory testing cannot duplicate field conditions in these cases, and for the efficacy of soil toxicants for termite control. However, others feel that laboratory test methods offer good screening tools for termite toxicants and have certain other advantages; for example, relative effectiveness of a product in controlling more than one pest can often be more easily evaluated in laboratory tests, variables are reduced, and specific species can be used in lab tests.

It should be noted that some species of termites do not colonize in soil but instead in the wood of housing and other structures, and some in other non-soil sites. Therefore, test protocols for such species will need to be developed that differ from the field stake method described in these guidelines. As suitable new test procedures are developed, the protocols will be incorporated into these guidelines.

Q. Public Comments on Test Methodology.

Through dispersal of early drafts of this subdivision, the Agency has already received comments from the public, and takes this opportunity to express appreciation for such submittals. Comments from organizations such as ASTM (American Society for Testing and Materials) and CSMA (Chemical Specialities Manufacturers Association) have been particularly useful in correcting and updating portions of the guidelines. However, to utilize fully some of the proposed changes in methodology, rationales and documentation supporting suggested changes should also be submitted. This could be done following publication of these guidelines. This supporting information will be reviewed and evaluated by Agency scientists in the process of making changes and improvements in the guidelines.

More supporting information on the following subjects already commented on is of special interest to the Agency:

1. Sanitizers - Carpet: Method No. 13 in § 91-30.
2. Sanitizers - Non-food contact surfaces: Method No. 3 in § 91-3.
3. Disinfectants - Efficacy against viruses (hard surfaces): Method No: 5 in § 91-30.
4. Disinfectants - Swimming pools: Method No. 14 in § 91-30.
5. Air Sanitizers: § 91-5.
6. In-can paint preservatives: § 91-54(a).
7. Metal-working fluids: § 91-54(b).
8. Fabric mildew fungistatic test: Method No. 1 in § 93-30.
9. Use dilution mildew fungicidal test: Method No. 6 in § 93-30.
10. Fabric, cordage, and fiber (rot, decay, mold and mildew method): in § 93-30.

R. Test Methods Needed.

The Agency is very interested in receiving copies of test methods that may ultimately be included or referenced in these guidelines. Also, references are requested that provide useful background information. Information on the following areas would be especially welcomed:

1. Test methods for egg sanitizers (see § 91-6);
2. Test methods on water purifier units (see § 91-8);

3. Test methods for controlling Legionaire's Disease bacteria in industrial water systems.

4. Test methods for controlling organisms that produce mycotoxins.

5. Test methods for controlling animal - or human-parasitic nematodes.

V. USDA COMMENTS AND EPA RESPONSES

FIFRA Sec. 25 (a)(2)(A) requires the Administrator to provide the Secretary of Agriculture with a copy of any proposed regulation at least 60 days prior to signing it for publication in the Federal Register. This provision is to permit the Secretary opportunity to comment on the proposal, and he did so for Subpart G, I, and J of the guidelines proposed in 1979, proposed when they were being developed as regulations. Since the present version of Subdivision G has been edited to reflect product performance recommendations and guidance the reader should consult CFR 40, Part 158 for current data requirements. References to performance standards in the Agency's response to USDA and SAP should be viewed as "suggested performance standards" for guidance purposes only. Specific comments of Dr. Flamm on Subpart G are published below. The page numbers mentioned in Dr. Flamm's comments refer to pagination in the June 22, 1979 draft reviewed at the time. For purposes of brevity in this addendum, each USDA comment is followed immediately by insertion of the corresponding EPA responses.

United States Department of Agriculture
Office of Environmental Quality
Washington, D.C. 20205
August 6, 1979.

Mr. Edwin L. Johnson (TS-769)
Deputy Assistant Administrator for Pesticide Programs
U.S. Environmental Protection Agency
Washington, D.C. 20460

Dear Mr. Johnson: Thank you for your July 5, 1979 letter, transmitting copies of EPA's proposed "Guidelines for Registering Pesticides in the United States." The guidelines transmitted include Subpart G - Product Performance; Subpart I - Experimental Use Permits; and Subpart J - Hazard Evaluation: Nontarget Plant and Microorganisms.

We are pleased that the U.S. Environmental Protection Agency is making progress in the development of guidelines for registering pesticides in the United States. These guidelines will be of value to those wishing to pursue registration of pesticides.

USDA cursory comments on each of the subparts are attached. We expect to have additional comments during the 90-day comment period after the guidelines are published in the Federal Register.

We waive the 30-day waiting period requirement for publication in the Federal Register after receipt of USDA comments as you requested in your letter.

We hope you will find our comments constructive. We look forward to continued cooperation with the U.S. Environmental Protection Agency as the development of these important registration guidelines progress.

Sincerely,

Robert C. Riley (for
Barry R. Flamm,
Director

USDA Comments on Draft of Subpart G

Subpart G - Product Performance

There is a wide disparity in performance standards between the three major classes of chemicals of interest to us. While we agree with the general concept of performance standards and think that they should be included in the guidelines, we feel that insecticide performance standards should be similar to the herbicide and fungicide and nematicide performance standards. In some instances, the insecticide performance standards may be beyond current scientific technology.

We also fail to understand how the insecticide performance standards were developed. Some of the major insects are not covered in the performance standards. There are different standards for some insects within a crop for no apparent reason. It appears that the performance standards for insecticides were decided in an arbitrary and capricious manner. We prefer to see an across-the-board performance standard of 70% as for the herbicides and fungicides and nematicides.

[EPA Response: The Agency is pleased to note the Department's concurrence in the general concept of performance standards for pesticides. Such standards, when utilized in direct relation to the tests procedures recommended in the appendices to these guidelines, should prove a major protection to the environment and the consumer, as explained more fully in the preamble parts I A and II B.

The Department is correct that performance standards for some section series are more explicit than for certain other section series. The Agency feels that more specific standards for some kinds of pest control products would be more useful, particularly in the case of fungicide, insecticides, and vertebrate control agents. Sections for the latter two groups already contain such specific standards. Perhaps general standards would be more suitable for products which control wide varieties and numbers of organisms at one time, such as preemergence herbicides. It would seem naive, however, to set, say 70% performance standards for products designed to control, for example, headlice, brown rot of peaches, nematodes in strawberry nursery stock, wood-rotting organisms, aflatoxin organisms infesting grains, rats, poison ivy, poisonous weeds, ticks, and pests of fabrics. A performance standard requiring only 70% control for uses such as these would not be tolerated by the user, consumer, or the marketplace. Buyers would never accept most food commodities receiving such a low level of control. Yet there are instances within each of these disciplines where 70% control might be tolerated and be considered reasonable acceptable, such a premergence control of crabgrass, control of tomato and potato late blight and control of algae in lakes. Thus, more specific performance standards linked to specific uses or site pest combinations would seem to be more appropriate direction, rather than more general standards.

With specific reference to comments concerning the invertebrate control sections (series 163.95), the insecticide performance standards used were supplied by experts in their respective disciplines throughout the United States. These experts include State, Federal, university, and industry scientists. Some of the performance standards were written in the American Institute of Biological Sciences (AIBS) performance series on efficacy test methods as part of an EPA contract to AIBS. (See Appendix to section series 163.95 (95-30(b)) for details of the test methodologies and standards).

In the areas of invertebrate control agents, such as control of mosquitoes, fleas, lice, ticks, biting flies, cockroaches, fire ants, wasps, hornets, poisonous spiders, scorpions, centipedes, and bedbugs, a performance standard of only 70% would be totally unacceptable. No significant public health protection would occur if a 70% performance standard were required in these cases. Similarly, 70% control for many insects attacking crops would result in commodities that would be unsalable and could neither be stored nor transported satisfactorily.

The Agency has explained in the preamble and emphasizes again that THESE PERFORMANCE STANDARDS MUST BE INTERPRETED PRINCIPALLY IN DIRECT RELATION TO THE TESTS AND TEST STANDARDS AS INDICATED IN THE GUIDELINES and should not be interpreted solely as applicable directly to the commercial or marketing areas. The latter may often demand higher performance standards than those mentioned in these guidelines. These performance standards should be used as suggestions for guidance purposes only.

The Agency welcomes USDA's able assistance in developing a list of pesticide uses and site/pest combination with corresponding realistic performance standards.]

Percentage reduction of a pest population is a good indication of efficacy, but does not entirely address the problem of crop protection. For instance, if there was a pest population of 10,000 individuals attacking a crop and the performance standard is 90% then 1,000 of these pests are still remaining which would be enough to cause economic damage. If the pest population was only 1,000 then 100 would be remaining and 100 may not be enough to cause economic damage to the crop.

[EPA response: The Agency recognizes this fact and discusses this problem in the preamble part II B. Generally, the percent reduction of a pest population is avoided whenever other more suitable measurements can be used instead, such as percentage of infested fruit, reduction in number of eggs present, reduction in the number of holes in leaves (or nuts or bolls), reduction in number of lesions on animals, and reduction in percent lodging.]

[§ 163.90-3 General Requirements]

Geographic distribution of tests is a good concept to ensure that the product will perform under a wide variety of climatic geographical conditions. However, we hope that EPA will use discretion with these requirements and not request data where the pest does not exist or the crop is not grown in significant acreage.

[EPA response: The Agency accepts the comments and now states in §163.90-3(b)(7) that the pesticide must be tested in each major geographic area where the pest is known to exist and be of importance, or where the crop is grown in significant acreage. When a pest control program is intended for only one locality where the pest is known to exist in significant amounts, test data from that locality are usually sufficient. Further instructions regarding testing in various georgraphic areas are located in the individual section series on efficacy.]

Steps should be taken to assure that the guidelines will not impede the development of data to register minor uses of pesticides and specialty chemicals such as attractants, hormones, pheromones or pathogens for use in IPM programs.

[EPA response: Specific standards for attractants, hormones, pheromones, and biological pesticides will be provided for in sections of the guidelines to be published in the future (Subpart M). As stated in the preamble at part II B, the performance standards are generally based on current agricultural practices in the United States, and are to be used as suggestions for guidance purposes only.

These standards do not take into account performances under any of the several integrated pest management (IPM) practices. However, if pesticides are to be considered as part of an IPM program, they must be sufficiently dependable to control designated pests, even though the amounts, frequencies, and timings may differ significantly from pest control under a non-IPM program. Therefore, it would seem logical to use pesticides that easily achieve satisfactory non-marginal pest control if one is to be confident of their use in an IPM program. Thus, the argument for lower performance standards for pesticides to be used in IPM program is not really very credible.

Periodic revisions of these guidelines will provide opportunity for greater attention to any such programs and any further developments as they become recognized practices. (See also preamble Part II. D.) The Agency will welcome comments on any means by which IPM standards could or should be incorporated into these guidelines.)

The Agency has updated information on IPM by adding to the Subdivision H discussion and to § 105-1(c), which states that "Labels for foliar treatments, soil treatments, greenhouse treatments, shade and forest treatments, uncultivated and non-agricultural area treatments, must include a statement which informs the user to consult with the local extension service or state university for information on economic threshold levels, timings, and pest management programs."

This is to alert such users to the possibilities of Integrated Pest Management Programs which may cover agricultural and non-agricultural sites.]

On page 244, reference is made to "Regardless of the site for this performance evaluation, the equipment, the equipment operation and adjustment, and procedure...must be identical to those employed in the spray drift evaluation study described...in Subpart J." It is impossible to have "identical" situations for this type of study. Also, our comment on Subpart J indicates that additional study is needed before finalizing these guidelines.

[EPA response: This sentence, which now appears in § 163.94-2(a)(5)(i), was changed to state that "aerial application performance evaluation SHOULD BE RUN IN CONJUNCTION WITH spray drift evaluation studies as described in section series §163.126 of Subpart J." As indicated in the Department's comments on Subpart J, the Agency is presently involved in discussions with the Department concerning spray drift studies.]

VI. FIFRA SCIENTIFIC ADVISORY PANEL COMMENTS AND EPA RESPONSES

FIFRA Sec. 25(a)(2)(A) requires the Administrator to provide the FIFRA Scientific Advisory Panel a copy of any proposed regulation at least 60 days prior to signing it for publication in the Federal Register. This provision is to permit the Panel opportunity to comment on the impact of this proposal on health and the environment. The specific comments of the Panel and the Administrator's reponses thereto on the June 22, 1979 draft are published below. In this instance, comments were supplied by Dr. H. Wade Fowler, Executive Secretary of the Panel, to Edwin Johnson, Deputy Assistant Administrator for Pesticide Programs. For purposes of brevity in this discussion, each Panel comment is followed immediately by insertion of the corresponding EPA response set off in brackets. Page numbers mentioned in Panel comments refer to the pages of the June 22, 1979 drafts under review at the time of the formal meeting on July 19-20, 1979. During this meeting, the proposed good laboratory practices sections of Subpart F were also reviewed by the Panel; however, all mention of the Subpart F material was deleted from the Panel's comments reproduced below to avoid confusion with the proposed Subpart G, I, and J presented and discussed in this issue of the Federal Register. The Panel's comments presented in this discussion consist of their general comments on this Subpart.

United States Environmental Protection Agency
Office of Pesticides and Toxic Substances
Washington, DC 20460
October 22, 1979.

Subject: Review of Proposed Guidelines for Registering Pesticides in the United States

From: Dr. H. Wade Fowler, Jr., Executive Secretary FIFRA Scientific Advisory Panel

To: Deputy Assistant Administrator for Pesticide Programs

The FIFRA Scientific Advisory Panel has completed review of Subparts G, I, and J of the Guidelines for Registering Pesticides in the United States. The Review was completed in open meetings held in Arlington, Virginia, during the period July 19-20, 1979.

Attached is a report of finding by the Panel.

The report was delayed due to a shift of office resources for resolution of Panel business relating to conclusion of the RPAR's on 2,4,5-T and Silvex. The secretary regrets the delay and sincerely hopes that the delay did not significantly impede progress with proposed rulemaking on the Guidelines. Please convey our special thanks to Dr. Preston, Mr. Jordan and all members of the EPA staff who participated in the meeting for an excellent briefing on the important features of the proposed rulemaking documents.

The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Scientific Advisory Panel completed review of several additional Subpart of Proposed Guidelines for Registering Pesticides in the United States during open meetings held in Arlington, Virginia, during the period of July 19-20 1979. The following specific subparts were reviewed during the meeting:

1. [illegible]
2. Subpart G. Product Performance.
3. Subpart I. Experimental Use Permits
4. Subpart J. Hazard Evaluation: Nontarget Plants [and Microorganisms].

Maximum public participating has always been encouraged at all meetings of the FIFRA Scientific Advisory Panel. In respect to this session of the Panel, a Federal Register Notice was published on July 3, 1979. In addition, telephonic notices and special mailings were sent to the general public who had previously expressed an interest in activities of the Panel. Written statements relative to the Proposed Guidelines, Subpart G, Product Performance, were received from Huntington Laboratories, Inc. In addition to comments by EPA staff, informal comments were received from the general public and representatives of the pesticide industry. The superb efforts of Dr. William H. Preston, Jr. and other technical staff of EPA, who presented various Subparts of the Guidelines to the Panel are worthy of special recognition. The Panel wishes to commend the Agency for being exceptionally cooperative in their working relationship with the Panel in all matters dealing with the proposed Guidelines. This has materially aided in the expeditious review of a large amount of technical material. Additionally, the Panel wishes to recognize the value of preliminary review of massive documents such as Subpart G, Product Performance (676 pages). Introduction of the document to the Panel and the public in Houston, Texas, during a special subcommittee meeting in November 1978 made it possible to focus attention on a limited number of central issues and avoid lost time in discussions emanating from problems of communication relative to issues easily resolved by Agency officials.

In consideration of all matters brought out in the meeting and a careful review of the proposed rulemaking document, the Panel submits the following report:

The FIFRA Scientific Advisory Panel is of the opinion that the... subparts of the Guidelines (Subparts ...G, I, and J) submitted to the Panel as proposed rulemaking during the Twenty-fourth meeting of the Panel, July 19-20, 1979, deals effectively with the intended procedures with a limited number of specific exceptions and recommendations:

I.....

II. Subpart G. Product Performance. This subpart is concerned with the effectiveness of pesticide products.

A. Section 163.95-1, Efficacy of Invertebrate Control Agents. The use of specific figures of required percent mortality and/or percent control is meaningless, and may impede adoption of Integrated Pest Management (IPM) programs. Panel Comments and Recommendations: The Panel wishes once again to object unanimously to the inclusion of specific figures for required percent mortality and/or percent control under the individual pests discussed in Section 163.95-1 "General Requirements for Invertebrate Control Pesticides." Very strong and virtually unanimous objections to this meaningless exercise were raised at the Houston Subcommittee Meeting (November 1978) by both the Panel and the attendees at this meeting. Inclusion of these precise and often unobtainable figures can only serve to cause endless discussion and contention between registrants and EPA. Moreover, the precise mortality or control data is dosage-related, is affected by genetic susceptability or resistance of pest races, by climatic conditions, and by method of application. Under Integrated Pest Management (IPM) it may be desirable to obtain lower degrees of control, e.g., 50-70% mortality, than are generally indicated in the Guidelines. This is important to protect food sources for beneficial insects. Major changes in this procedure have been made in the revised guidelines under Section 163.93-3, Performance Standards Acceptable Levels of Pest Control (for Fungicides and Nematicides) where the phrase, "the product must provide, under moderate to severe pest pressure, at least 70% control of the pest organisms or their symptoms, (compared with untreated controls)." Lesser claims, such as, "aids in control" or "suppresses" may be made if less than 70% control of the plant disease is obtained. Another appropriate phrase would be, "the product must provide good commercial control of the pest organisms or their symptoms." This phrase would apply specifically to either standard pesticide usage or to IPM usage. The Panel recommends that this more appropriate, and clearly much more acceptable, terminology be applied to Section 163.95-1, "General Requirements for Invertebrate Control Pesticides" and elsewhere in the guidelines where appropriate.

[EPA Update: It should be noted again that these SAP comments reflect a 1979 draft of Subpart G written for proposed regulations. This current Subdivision G is written for guidance purposes only, and the performance standards are to be used as suggestions in areas covered by the waiver of efficacy data submission policy (See Part I.B. of this Discussion).]

[EPA response: Responses to the same comments made by the U.S. Department of Agriculture appear in Part V of this discussion. A more extensive explanation of performance standards is now provided in the preamble Parts I C 3 and II B 1. Attention should be focused specifically on the interpretation of performance standards: they should be utilized in conjunction with the test standards and recommended test procedures of the Appendices and should not be interpreted exclusively as applicable to the commercial arena.

The suggested test procedures require applications at the rate or rates specified on the label of the proposed product. Such rates are the ones expected to perform satisfactorily regardless of genetic susceptibility, pest resistance, climatic conditions, and methods of application if not already specifically limited on the pesticide label.

The Agency has also addressed comments relating to IPM in Part V of this discussion indicating the it would deal separately with the special considerations required for IPM, biological pesticides, pheromones, hormones, attractants.

The Agency is particularly concerned about the inadequacy of general performance standards such as 70% control for all plant disease and pest control products, and is hoping to obtain during the public comment period more specific and realistic performance standards in these areas.

The Panel's suggestion that the performance standard by "the product must provide good commercial control of pest organisms or their symptoms" is not particularly helpful, since such a criterion would likely require much higher percentages in performance standards than are provided in the proposed guidelines, percentages that would often be unobtainable using the test procedures suggested in the Appendix. Commercial marketing procedures would not likely tolerate much less than 98-99% pest control or control of pest symptoms for most crop produce that must be shipped long distances or stored for more than very short periods.]

B. Section 163.96, Efficacy of Vertebrate Control Agents. The use of specific figures for required percent mortality and/or percent control is meaningless, and may impede adoption of Integrated Pest Management (IPM) programs. Panel Comments and Recommendations: The Panel reiterates the general comments and recommendations previously outlined for Section 163.95, Efficacy of Invertebrate Control Agents.

[EPA response: The Agency reiterates its position as explained under A and in EPA update (above), in Part V of this discussion, and in the preamble Parts I C 3 and II B 1.]

[End of comment.]

SUBDIVISION G -- PRODUCT PERFORMANCE

Series 90: OVERVIEW, DEFINITIONS, AND GENERAL CONSIDERATIONS

§ 90-1 Overview: product performance.

(a) General concepts. (1) The term "product performance" refers to all aspects of a product's effectiveness and usefulness. Typically, any evaluation of product performance is conducted in light of expressed and implied labeling claims or recommendations concerning pests, sites, methods of application, application equipment, dosage rates, timing and number of applications, use situations, nature and level of pest control, duration of pest control, compatibility with other chemicals, benefits and/or adverse effects of product use, compatibility of common practices associated with the sites, active ingredient status of chemicals in the formulation, and equipment.

(2) Typically, initial laboratory, greenhouse, or small plot field testing is conducted to determine the effectiveness of a substance to control or kill specific pest organisms, or to produce desired effects on plants or plant parts, and also to determine whether the substance has sufficient pesticide potential to warrant larger scale testing.

(3) Effectiveness and usefulness of the proposed product is further proven through advanced large-scale laboratory tests, field tests, in-use tests, or simulated-use tests by procedures which closely approximate actual use and which employ typically-used application equipment.

(4) All advanced tests must address those factors which would normally be encountered in the use patterns claimed for the product. These factors would depend on the type of pest and site to be treated, and may include: specificity, degree, and duration of pest control; impact of climate on chemical residuals and bait acceptance; nature and extent of spray coverage; adverse environmental effects such as bioaccumulation (see Subdivision N), and toxicity to beneficial nontarget organisms (see Subdivision E, F, J, L, and M); increase in population levels of other pests of the target site resulting from control of predatory or competitive microorganisms, or interference with the performance of other pesticides; or any other factors which would establish the safe, effective use of the product.

(5) Except as provided in 40 CFR Part 172, an experimental use permit must be obtained to cover test trials involved in use of a pesticide that is not registered with the Agency and test trials involving a new use of a previously registered pesticide. (See Subdivision I for further information on experimental use permits.)

(6) The Agency may require additional information on benefits when a product does not achieve the performance standards, when exorbitant rates are used to achieve the performance standards, or when exceptionally low levels of effectiveness are attained.

(b) FIFRA mandate and waiver policy. To register a substance as a pesticide product, FIFRA Sec. 3 requires an applicant to submit data to show that the product will perform its intended functions without causing unreasonable adverse effects on the environment. Performance tests are necessary, therefore, to show that products to perform their intended function. However, § 3(c)(5) of the amended FIFRA provides that "in considering an application for the registration of a pesticide, the Administrator may waive data requirements pertaining to efficacy, in which event the Administrator may register the pesticide without determining that the pesticide's composition is such as to warrant proposed claims of efficacy."

As previously discussed, in 1979 the Agency issued regulations implementing several provisions of the 1978 FIFRA amendments (44 FR 27933, 40 CFR, Part 162, May 11, 1979). Among the provisions implemented was the efficacy data waiver authority provided by Sec. 3(c)(5) of FIFRA. The Agency defined in § 162.182(d) the circumstances with efficacy data were required to be submitted as a matter of course. Other requirements that efficacy data be submitted were generally waived. The Agency is proposed in the Regulations for Registration, Reregistration and Classification Procedures (47 FR 40659, 40 CFR, Part 162, September 15, 1982) to extend the efficacy data waiver to additional use patterns, and it will include all registration actions, both conditional and unconditional. As stated in its previous waiver, the Agency's primary mandate under FIFRA is to evaluate the health and safety aspects of pesticides. Submission of efficacy data will be waived for all uses of pesticides except those where control cannot reasonably be observed and determined by the user and lack of control results in a clear adverse health effect; or certain pesticides under suspension or cancellation orders, or in cases where lack of efficacy is reported; or in evaluation of benefits when substantial risks have been identified; or when factors exist that make submission of such data necessary to support the presumption that it is efficacious. The specific uses that require efficacy data are specified in 40 CFR Part 158 - Data Requirements for Registration § 158.32 with references to the testing methodology and and protocols in Subdivision G - Product Performance. The level of label claims are described in Subdivision H - Labeling Guidelines for Pesticides Use Directions (§§ 100 thru 106) and guidance is also given to test methodology needed to support these claims when necessary. Details of the extension of the efficacy waiver are:

(1) With regard to public health uses, the Agency has determined that a threat to human health exists when the presence of the pest organisms cannot be readily observed by the user. Such uses include, the following ones listed and product performance data are, therefore, required to be submitted on products having:

(A) Uses of antimicrobial agents intended to control pest microorganisms (except bacteria, pathogenic fungi, or viruses living on or in man or other animals) that pose a threat to human health and whose presence cannot readily be observed by the user, including, but not limited to, microorganisms infectious to man in any area of the inanimate environment;

(B) Uses of fungicides intended for control of organisms that product mycotoxins.

(ii) With regard to certain pesticides under suspension or cancellation action or subject to a Notice of Special Review under Section 162.11(a)(3)(ii), the Agency has determined that product performance data are required to be submitted to support any new or additional uses of products that are:

(A) Already under suspension or cancellation action by the Agency for reason that some uses cause human or environmental safety hazards, provided that the risks identified in the suspension or cancellation action also apply to the new uses; or

(B) Already subject to a Notice of Special Review, provided that the risks identified in the Notice also apply to the new or additional use(s).

(2) For those products for which the Administrator will ordinarily waive the requirement for submittal of efficacy test data as indicated in paragraph (b)(1) of this section, the Agency reserves the right and authority to require, on a case-by-case basis, submission of such efficacy data or other test data for any specific product, registered or proposed for registration, whenever the Agency deems that such data are necessary to make proper evaluations for a decision as to acceptability for registration or continued registration.

(i) With regard to conditions where the Agency may request efficacy data but not be limited to these causes for any product, registered or proposed for registration such as when:

(A) A lack of efficacy has been reported for it; or

(B) The Agency needs such data to evaluate benefits of the pesticide (or of alternative pesticides) when substantial risks have been identified; or

(C) Factors exist that make submission of such data necessary or desirable to support the presumption that it is efficacious.

(ii) Thus, the guidelines in this Subdivision shall be used by registration applicants as efficacy testing standards in conjunction with label claims and efficacy data reporting requirements when efficacy data must be submitted to support registration applications; in addition, the guidelines should also be used to provide helpful guidance on testing to determine the claims and directions for use on labeling to products for which efficacy data submittal is waived.

(c) Relation of general considerations to specific considerations. (1) The provisions contained in § 90-3 General considerations apply to most pesticides except antimicrobial-type pesticides. To understand the product performance considerations fully, the registrant should read § 90-3 along with the appropriate sections dealing with specific kinds of products. Because of their unique and complex use patterns, antimicrobial type pesticides are covered in §§ 91-1 through 91-8 which contain both general and specific requirements for these pesticides.

(2) The actual test procedures will vary according to the characteristics of the chemical, the type of formulation, the target pest, the use patterns, the methods and timing of application, and many other factors. But certain basic techniques are essential, and these are discussed in § 90-3 General considerations: product performance. The relationship of test results to label directions for use and limitations is described in §90-3 and in sections on specific uses elsewhere in this subdivision. The applicant or registrant should study these appropriate sections as integrated parts of the general and specific guidelines for Subdivision H - Labeling Guidelines for Pesticide use Directions.

(3) Identification of explicit methods in these guidelines for all uses is not practical, because an acceptable test for one product or use may not be valid to support another product intended for the same use, or for the same product intended for even a slightly different use. Examples of acceptable methods are provided in specially designated sections of the guidelines, but the applicant or his testing agent must be responsible for the validity of any particular test or tests employed.

(d) Relation of Subdivision G to other subdivisions of the guidelines.

(1) Relation to 40 CFR Part 158 Subparts A and B. The registration applicant is referred to CFR 40 Part 158 -- Data Requirements for Registration for general provisions and policies pertaining to registration data requirements including policy on flexibility in relation to deviation from test standards and acceptable protocols, when tests are required (§158.32). and requirements for additional data.

(2) Relation to Subdivision H. The registration applicant is referred to Subdivision H for guidance on pesticide labeling. Label claims in Subdivision H should meet the performance standards in Subdivision G when applicable. An important objective of the testing programs described in Subdivision G is to develop sufficient data to support appropriate and adequate efficacy related labeling claims and directions for use. The applicants should read the general and appropriate specific sections of Subdivision H before initiating efficacy testing in support of Subdivision G.

(3) Relation to Subdivision I. An experimental use permit may be required for trials involving use of a pesticide that is not registered with the Agency and test trials involving a new use of a previously registered pesticide. These permits are issued in advance of proposed field studies after submitting proper applications for the Permits. For specific details and information on Experimental Use Permits, see Subdivision I.

(4) Relation to Subdivision J. The registration applicant is referred to Subdivision J Hazard Evaluation: Nontarget Plants for data guidance on pesticides that contact plants through direct application or through movement of pesticides in the environment, and submission of data on adverse phytotoxic effects to nontarget plants both within the target area and outside the target area.

§ 90-2 Definitions.

Terms used in this subdivision shall have the meanings set forth in FIFRA, at § 162.3 of the FIFRA Sec. 3 regulations, at § 302, and at § 60-2. In addition, for the purposes of this subdivision:

(a) The term "effective dosage range" or "EDR" refers to the range of dosage levels beginning with the lowest dosage capable of achieving the level of control specified by the applicable performance standard for the least taxing conditions under which it will be used (e.g., pest levels, soil types, water conditions, geographical and climatological conditions, etc.), and ending at the losest dosage required to achieve the specified level of of control under the most taxing conditions under which it will be used.

(b) The term "effectiveness" refers to a product's ability to control the specific target pest or produce the specified plant or animal response when the product is applied in accordance with the label directions directions, precautions, and limitations of use. The term "effectiveness", as used in this subdivision, is synonymous with the term "efficacy".

(c) The term "full coverage", as used in common agricultural practice, refers to a volume of spray applied to plants to the point of runoff or drip.

(d) The term "large scale plot" refers to any plot large enough to permit the use of typical commercial application equipment (when such equipment is needed for pesticide application).

(e) The term "low volume" or "LV", as used in common agricultural practice, refers to a total volume of spray applied broadcast at more than 0.5 gallon but less than 5.0 gallons per acre (more than 1.89 liters but less than 18.93 liters per hectare) 1/ or less than a full coverage spray.

(f) The term "minimum effective dosage" or "MED" refers to the lowest dose level at which the test substance achieves the level of control specified by the applicable performance standard.

(g) The term "performance data" refers to any data pertaining to pesticide effectiveness and usefulness.

(h) The term "serial application" refers to the label recommended use of a pesticide on a site before or after application of another pesticide to that site, such that the presence of one of the pesticides may affect the effectiveness and usefulness of the other.

(i) The term "ultra low volume" or "ULV", as used in common agricultural practice, refers to a total volume of 0.5 gallon or less per acre (1.89 liters or less per hectare) broadcast. 1/

1/ For further information, refer to the American Society of Agricultural Engineers' Pesticide Application Subcommittee of the Agricultural Chemical Application Committee. 1978. Uniform terminology for pesticide spraying ASAE Handbook, ASAE-S327 (Agricultural Engineers Yearbook), p. 313.

§ 90-3 General considerations.

(a) Scope of considerations. (1) The registration applicant is reminded that certain efficacy data submittal requirements are waived as described generally in § 90-1(b) of this subdivision. Therefore, while the other paragraphs in this section and the other sections in this subdivision establish requirements concerning the methodology of efficacy testing and the content of test reports, they do not independently establish any data submittal requirements.

(2) The standards contained in this section apply generally to all studies in this subdivision [except for antimicrobial agents (§§ 91-1 through -8)], unless another section of this subdivision contains a specific standard on the same subject. In such a case, the specific standards in the other sections shall apply to the conduct of that particular study.

(b) Test standards. (1) Personnel. All testing and evaluation should be done under the direction of personnel who have the education, training, and/or experience to perform the testing and evaluation in accordance with sound scientific experimental procedures. The Agency may require resumes of personnel who have performed, supervised, reviewed, or evaluated the testing.

(2) Test substance. (i) The test substance shall generally be the formulated product.

(ii) In addition to or in lieu of data otherwise mentioned by this subdivision, the Agency may require, after consultation with the applicant, data derived from testing to be conducted with:

(A) An analytically pure grade of an active ingredient with or without radioactive tagging;

(B) The technical grade of an active ingredient;

(C) The representive technical grade of an active ingredient;

(D) The inert ingredient of a pesticide formulation;

(E) A contaminant or impurity of an active or inert ingredient;

(F) A plant or animal metabolite or degradation product of an active or inert ingredient;

(G) The end-use pesticide product;

(H) The end-use pesticide product plus any recommended vehicles and adjuvants;

(I) Any additional substance which could act as a synergist to the product for which registrations is sought; or

(J) Any combination of substances mentioned in paragraph (b)(2)(ii) of this section.

(3) Dosage rates. (i) Typically, the test substance should be tested at various dosage levels including the dosage rates associated with the proposed use. Dosage rates should be tested as requested by each section. Special attention should be paid to treatment rates on food crops in relation to the tolerance or proposed tolerance. Additional guidance on selection of test dosage rates may be found in the specific discipline sections of this subdivision.

(ii) The test program (sum total of preliminary and final stage tests) should establish clearly the effective dosage range (EDR) or the minimum effective dosage (MED) as appropriate for the uses involved. The development of an EDR, rather than a single MED, is encouraged, whenever feasible and practical, because the EDR permits at least some of the users the opportunity to use rates other than the maximum rate needed to cover all use situations.

(4) Serial applications. The label use directions may specifically direct serial applications of different products, such as when tank mixing is impractical. These directions should be supported by tests designed to compare the effectiveness and usefulness of application of each product alone with applications of the products applied serially. Special emphasis should be directed toward determining whether or not a minimum time interval between applications of the respective chemicals is warranted.

(5) Package mixtures. These products contain more than one active ingredient. Data are needed to establish the efficacy of each active ingredient in a package mixture. However, since it is the efficacy of the formulated product/use-pattern combination that is to established, testing as part of an experimental use permit program is usually conducted with the package mixture only. In many instances one or more of the separate active components will have been previously registered as a single-component product. In such cases, these data may be included as part of the data base for the package mixture when suitable comparability data have been developed to demonstrate that each active ingredient is effective and safe to use on the target site regardless of whether it is used alone or in the package mixture. Note: package mixtures which result in a significant amount of inappropriate or unnecessary usage (dosages, certain active ingredients, inappropriate timing, and unnecessarily high number of applications) of one or more of the active ingredients are not acceptable.

(6) Tank mixes. Product labeling which implies or recommends mixing products in the spray tank before application should have acceptable supporting data as described below.

(i) Directions for tank mixing of products should be supported by performance data on each component (of the proposed mixture) tested separately as well as data on the mixture used at the dosage rate(s) specified for each pest indicated. The combined minimum and combined maximum dosage rates of each product in the tank mixture should be tested. [Guidance pertaining to tank mixtures for environmental fate data appear in § 164-4(4) of Subdivision N.]

(ii) The components of a pesticide tank mixture should be physically and chemically compatible. Evaluations of physical compatibility should be conducted using maximum rates of each component in minimum recommended volumes of diluent per acre or hectare, demonstrating effects of order-of-component-addition to the tank, and evaluating effects of water hardness, pH, and temperature on separation, suspendability, and sprayability. Where compatibility is questionable from static tests, actual testing should be done which should employ constant agitation of the mixture as in most commercial field sprayers. Note: tank mixes which result in a significant amount of inappropriate or unnecessary usage (dosages, certain active ingredients, inappropriate timing, and unnecessarily high number of applications) of one or more of the active ingredients are not acceptable.

(7) Adjuvants. Products with labeling which allows or recommends the additions of separately packaged adjuvants to the spray tank should be supported with data indicating their benefits (if claimed) and any detrimental effects (such as increased crop phytotoxicity) which may result from their addition to the herbicide, plant regulator, desiccant, or defoliant. The only adjuvants actually permitted for use with a pesticide will be those adjuvant brand names or defined adjuvant classes specifically named on the pesticide label. The adjuvant rate or range of rates should be named on the pesticide label. The adjuvant rate or range of rates should be indicated on the pesticide label, and should be supported with data on efficacy and any detrimental effects. If a range of adjuvant rates is recommended, the maximum and minimum rates within that range should be evaluated in conjunction with the intended pesticide product. (Additional details on adjuvants are discussed in § 94-2(a)(9)).

(8) Geographic distribution of tests. Pesticide products marketed on a nationwide basis may be used under a wide variety of conditions. The pesticide should be tested in each major geographic area where the pest is known to exist and be of importance, or where the crop is grown in significant acreage. When a pest control program is intended for only one locality where the pest is known to exist in significant amounts, test data from that locality are usually sufficient. Further instruction regarding testing in various geographic areas are located in the individual section series on efficacy.

(9) Test design and statistical procedures. (i) General test design. Sound statistical designs and procedures are necessary to assure that valid and appropriate statistical analyses of the data can be performed and that any observed statistically significant differences are attributable to the respective pesticide treatments, and that such pesticide treatments provide the expected pest organism response. Direct comparison of group means of treated sites and untreated control sites is usually sufficient for evaluating treatment effects. The test results, however, for most pesticidal purposes, should show more than just statistically significant differences between treated and control sites: the differences should generally be of a magnitude which meets or exceeds the performance standards described in each of the subsequent sections of this subdivision. [See paragraph (d) of this section for more information on performance standards.] For useful references on test design and statistical procedures, see references 2-5 in § 90-30.

(ii) Multiple site and pest target combinations. When more than one pest or site is involved in pesticide applications, separate tests are usually necessary to evaluate product performance against each kind of pest or each kind of plant under each set of variables or use conditions. For vertebrate control agents, however, it is preferred that efficacy be evaluated on one pest species at a time. If more than one method of application is to be employed (such as, for example, air and ground sprays, drenches and injections, or impregnation and surface coating), experiments should be designed to obtain the required data for each method, on or in the same experimental sites, and if separate evaluations can be made, their respective levels of control can be assessed during the test.

(iii) Replicates. Generally, the number of replicates necessary to demonstrate treatment differences will depend upon several factors, such as variability of test organisms and materials (crops, pests, application equipment, soil conditions), magnitude of treatment effects, and the desired statistical confidence level.

(iv) Sampling procedures. Sampling procedures should assure that all of the characteristics of the test population to be measured are represented in the samples. The size and number of samples necessary for reliable estimates will vary mainly with the level and uniformity of the organism or the effect to be measured as well as the size and precision of available equipment. For example, entire replicates may need to be harvested to make accurate yield estimates or to measure plant growth responses when low pest populations are present, while representative portions of each plot will probably be sufficient for measuring high density pest populations.

(v) Considerations for crop test designs. In designing the test, all variables, both uncontrollable (such as soil texture and microclimate) and controllable (such as irrigation, cultivation, pruning, fertilization, cultivar, and test product application) should be considered. Care should be taken to duplicate carefully all controllable variables (other than test product application) on all treated and untreated plots.

(A) Plot sizes. Plots should be large enough to reflect actual use conditions and to allow representative application techniques, which may include commercial application equipment. Small plots, such as a single 10-ft (3.3m) row of vegetable and forage crops, and single-branch or single-tree plots of fruits, are ordinarily insufficient to support valid conclusions about product performance. Factors such as the crop grown, equipment used, expected incidence of the pest, need for residue samples, yield data, and quality studies should be considered in selecting the size of field plots. The plots should be located within a field so as to be representative of conditions throughout the field. Areas of fields such as borders and atypical wet or dry locations must generally be avoided, unless these are the optimum areas for pest damage. More specific guidance as to adequate test plot sizes is provided in the sections on specific pesticide types which follow this subpart.

(B) Crops or sites treated. A representative number of the major cultivars of crops should be represented in the tests. Cultivars should be more extensively utilized as a test variable to demonstrate adverse responses in tests.

(C) Climatic factors. The testing schedule should be designed to permit evaluation of the effectiveness of pesticides applied under different environmental conditions such as low versus moderate and high precipition, cool and cloudy versus normally hot and sunny conditions, and low versus high humidity and dew formation, as appropriate to the product use.

(D) Edaphic (soil) and other substrate factors. The effectiveness of a pesticide product can often be influenced by the type of substrate to which it is applied. Therefore, testing procedures should be designed to evaluate product performance on those surfaces or substrates intended for treatment. A number of variables relating to soil, such as soil temperature, texture, fertility, pH, organic matter content, moisture content, tillage practices, irrigation, and crop rotation schedules, measurably influence performance of soil-applied pesticides. Accordingly, field tests should be designed, as appropriate, to assess the potential effects of the pertinent variables. Data to determine safety to crops planted in the treated area the same season and in following seasons should be submitted. [See § 121-1 of Subdivision J on phytotoxicity and §§ 165-1 and -2 of Subdivision N.]

(E) Spray volume. The volume of spray is another important variable affecting the performance of pesticides because it directly relates to the distribution and coverage obtained on the target site. When appropriate, testing procedures must be designed to determine the acceptable range of spray volume to be used with the intended application equipment. Special emphasis should be placed on obtaining data on the minimum and maximum spray volumes when a specific amount of product is intended for use in a range of spray gallonages.

(F) Timing of applications. Test reports should specify the time at which treatment was begun, duration of exposure (if applicable), and intervals between succeeding applications. For example, data on crop treatments should include the following information (when applicable) in relation to timing of applications:

(1) Date(s) of treatment(s) and harvest.

(2) Treatment time in relation to number of days before or after planting, plant emergence, or harvest.

(3) The stage of growth of the crop when treatment was made.

(4) The stage of growth or expected appearance of the pest at treatment time.

(5) Duration of exposure to pesticide treatment.

(6) Treatment-to-observation interval(s).

(G) Seasons. For pesticides used outdoors where variations in climatological conditions alter efficacy, the seasons during which the tests were conducted shall be reported.

(c) Suggested performance standards. (1) When efficacy data submissions are not waived, performance standards will represent the levels of product performance that are exhibited by pesticides on specific site/pest combinations and considered acceptable for registration purposes. These standards, however are not always absolute or inflexible. Labeling statements (claims) described in Subdivision H are based on the performance standards and utilize tests in Subdivision G. Data showing deviation from the standards, however, may prompt the Agency to request additional information on benefits (e.g., increased yields, unblemished fruits, reduction in nuisance pest levels, etc.), such as from the use of high rates to achieve standards greater than stated in the guidelines, or from low levels of effectiveness in relation to wasteful applications and unnecessary pollution of the environment.

(2) The suggested performance standards alone do not reflect the entire perspective of what is expected regarding a pesticide's effectiveness. To meet a performance standard, a pesticide would frequently be expected to control pests when they are at levels that cause economic damage (such as to crops). Demonstration of (say) 70% control when pest numbers are far below economic injury levels would not be adequate proof of efficacy.

(3) Suggested performance standards are usually expressed as percentages of pest control, or percentages of other intended responses, calculated from measurements made on treated plots compared with those made on untreated control plots. Reliance only on untreated control plots, however, is not always sufficient or appropriate, particularly when testing on very large areas against mobile pests, when testing dog repellents, or when conducting tests in mobile substrates, e.g., herbicides applied to moving bodies of water. In such cases, a product may be tested against some other base, such as against another formulation or chemical of known efficacy, or by comparison to pest levels or damage measured before and after the test. For most pest control patterns, efficacy data should be obtained under a full range of pest severity conditions, with particular emphasis on the maximum pest severity likely to be encountered by users.

(d) Adverse effects. To the extent possible, efficacy tests should be designed to evaluate possible adverse effects resulting from use of the pesticide. The following are examples of adverse effects which should be considered:

(1) Phytotoxicity. Details of data submitted on phytotoxicity are provided in Subdivision J of these guidelines, but the following explanation may serve as an introduction for those persons developing product performance data. A good test design providing for dosages higher than necessary for pest control on plants will allow an estimate of the adequacy of the margin of safety between effective pesticide levels and those which may injure the plants intended to be protected. Phytotoxicity is usually measured in terms of chlorosis, malformed plant parts, leaf burning, plant wilting, stunting (reduced height), reduced stand, and death. For certain uses, some injury can be tolerated (depending upon the reversibility of effects, or on economic or aesthetic factors), but all injuries should be evaluated and reported. Accordingly, the lack of observable phytotoxic effects should also be reported.

(2) Effects on quality of commodities and inanimate objects. Test programs should be designed to evaluate adverse pesticide effects on treated commodities and surfaces, such as discolored and weakened fabrics, deteriorated food quality, decrease in wool quality, milk production, and unsightly residues on plant plant foliage. Taste panel tests, color determinations, blemish counts, livestock palatability trials, or other similar measures, should be considered for incorporation into the test program, depending upon the end use of the commodity. For information on sensory methods of

evaluation (flavor, odor, taste, palatability, and repellency), see reference 1 in § 90-30.

(3) Yields and other effects. Such determinations will aid in advanced planning in the test design. Pesticide treatments may decrease yield, reduce crop quality, or so alter the normal ripening or maturing process that economic problems arise in harvesting. Data should address the absence or the extent to which such effects occur.

(4) Effects on wildlife and aquatic organisms. Refer to Subdivisions E and L of these guidelines for test requirements to evaluate adverse effects on wildlife and aquatic organisms. Observations and evaluation of efficacy test results should include relevant information about possible increase in harmful nontarget organisms as a result of the pesticide use and application, as well as possible increase in beneficial organisms to intolerable levels, and possible adverse effects of presence of pesticides. Treated dead and dying pest organisms as potential food for domestic or wild nontarget organisms.

(e) Test descriptions and data reporting. (1) Extent of report. When applicable, systematic and complete descriptions of the tests employed and accurate reporting of data derived from laboratory tests and field tests to support label claims for performance of a product or mixture may be essential for proper Agency review and evaluation. Both English and metric units should be given for all rates and measurements for laboratory tests; English units alone are sufficient for field data, but both measurements may be supplied. Application rates expressed as "ppm" must indicate the basis (weight/weight or weight/volume).

(2) Assembly of report. Considerations for assembling the reported efficacy data to expedite Agency review of the detailed report include:

(i) An index of the test reports arranged primarily according to the general types of performance data and secondarily by the site/pest combinations on the label. Additional subdivisions based on methods of application, soil textures, geographic areas, or other pertinent variables are encouraged wherever it is feasible and will facilitate an evaluation of the data. It is recommended that numbered tabs be used to identify the individual test reports.

(ii) Tabular summaries of the data. It is recommended that each horizontal line (or series of several lines) be equivalent to a test, and that columns reflect the major test variables being reported, such as those details listed in paragraph (e)(3) of this section. These summaries should be organized primarily according to site/pest combinations and secondarily according to pertinent variables, such as methods of application, soil textures, or test locations. The purpose of summary tables is to present a condensed and simplified overview of the scope of the test program and the level of product performance obtained. In order to achieve maximum utilization of space in tables, the use of abbreviations, acronyms, or defined codes as well as the grouping of several types of information within columns is encouraged.

(iii) Summarized conclusions related to label claims. Data analyses and evaluations should be included.

(3) Details of report. All details of the tests should be reported, giving particular emphasis to variables that relate to the label directions, limitations, and precautions. Such details may include:

(i) Personnel data. Names, positions, and addresses of persons who conducted and supervised the tests should be reported. Names of all persons who recorded or generated data for the tests should be made part of the record (not submitted but held as records) along with the dates when the items of data were recorded.

(ii) Test substance. (A) Identification should be made of the test substance, including chemical name, molecular structure, and qualitative and quantitative description of its chemical composition as required by Subdivision D of these guidelines.

(B) Manufacturer and lot sample numbers of the test substance should be reported.

(C) Type of formulation and content [percent and, for liquids, pounds per gallon (kg/l)] of active ingredient should be reported. When a product is diluted before or during application, the report should specify the quantities and identification of each diluent.

(D) For many pesticide products, data on similar formulations may be used to supplement data on the specific formulation when the comparability of the formulations has been demonstrated. This procedure is not acceptable, however, for vertebrate control agents because very slight differences in formulation may cause marked differences in efficacy due, principally, to the highly developed sensory perception of many vertebrate pests.

(iii) Testing period. Report dates during which each test was conducted.

(iv) Method of application. The methods and types of pesticide placement, such as surface, sub-surface (as in soils), or incorporated, shall be reported. Descriptions of surface placements should indicate the method of application, such as wiping, soaking, mopping, dusting, painting, spraying, trail-building, broadcast seeding, or scoop placement.

(A) If the surface is to be sprayed, details on spray volume, such as ultra low volume (ULV), low volume (LV), concentrate, or conventional full coverage spray, should be given in terms of volume per unit area.

(B) For sub-surface methods, descriptions should indicate whether done by furrow placement, side-dressing, injection, or burrowbuilding. When describing injection methods, such information as spacing, number, and arrangement of chisels with respect to the row and depth of injection should be given.

(C) Descriptions of incorporated methods of application should indicate whether done by mixing, drenching, or impregnating. [For the latter case, pursuant to § 162.4(b) and (c) of the FIFRA Sec. 3 regulations, impregnated articles for which pesticidal claims are made or implied as to protection of other surfaces or objects are considered to be pesticides.] Details on the methods and equipment used and depth of incorporation may be requested for agricultural applications to soils.

(D) Information detailing the type of application, such as row (furrow placement, band, or side-dressing) or broadcast, bait boxes, swath placement, pressure-treated, or soaked, should accompany the test report data. Pesticides applied as row or band treatments should specify the band width and the amount of material used per unit of linear row distance, and the amount of material per acre (or hectare) and the row spacing. When per acre (hectare) figures are included in test reports for row or band treatments, the report should specify whether these figures represent the "actual" amounts of pesticides applied or the "broadcast equivalent" rates.

(v) Equipment. (A) The types of equipment used should be reported. This may include such items as mistblowers, cyclone seeders, hydraulic ground sprayers, artificial perches, dusters, burrow builders, soil injectors or incorporators, aircraft systems (specify whether fixed wing or helicopter), metering devices, smoke generators, rodent guns, impregnators, and aerosol dispensers.

(B) For mistblower applications, information on spray volume, air velocity, swath width, distance from nozzles to the target, and angle of air flow from the vertical should be given.

(C) For pesticide application through irrigation systems, the information includes: the types of systems used, such as sprinkler (stationary or mobile), furrow, drip, or flood; the quantity of water applied; description of the pesticide metering devices; pesticide concentrations in water samples collected at various points throughout the system; and any spatial arrangement of crops (if applicable).

(D) When pesticides are to be mechanically incorporated into soil, information should be reported on the equipment used, speed and depth of operation, number of passes over the treated area, and intervals between repeated incorporations (if applicable). Sufficient information on mechanical incorporations into soils or other growth media used at sites other than fields may be needed.

(vi) Dosage rate. (A) The dosage rate expressed as active ingredient and formulated product should be reported. Dosages should be reported in terms similar to the following: amounts per unit of surface area, per unit volume of solvent or diluent, per unit volume or weight of commodity, per unit volume of space, per unit area and depth (acre-foot or hectare-meter), per linear distance of crop row, per animal, per unit weight of animals, and the length of time of spraying and the distance from the target surface (as for certain pressurized products).

(B) When other pesticides are applied in the test area, the rates of application for each product, the identification of pesticides used, and the timing of application should be reported.

(C) Spray volumes should be included in the data reports.

(vii) Description of application site. (A) The site of application should be reported.

(B) The following information should be taken into consideration in describing the sites. Contact pesticides are applied to the pests themselves or to plant parts for which plant regulator, desiccant, or defoliant activity is intended. Residual pesticides are applied to substrates or surfaces which will in turn be contacted by pests. The specific site of application required for effective use of residual pesticides may be related to feeding or behavioral habits of insects and animals, characteristics of plants, mode-of-action of the pesticide, or the location of infection sites of plant pathogenic fungi and bacteria. For example, dogs establish urinary scent posts, and certain repellents should be applied to these areas to be effective; or, as another example, since mites feed principally on the undersides of plant leaves, nonsystemic miticides should be applied to both surfaces to control those feeding and those crawling around. The site of application may also be directly related to the mode-of-action of the pesticide. A systemic insecticide may, for example, be applied to foliage or the root system so it can be transported to various parts of the plant where it will kill or repel feeding insects. A plant regulator may be sprayed on the foliage and cause the desired response at parts of the plant that were not directly sprayed. A herbicide may be applied to the soil, be absorbed by the roots, and be translocated through the stem to the foliage where it exerts its pesticidal action.

(C) Texture of soil and its organic matter content should be reported if applicable to the pesticide usage. In situations where pesticides are intended to act through soil or in burrows, conditions such as tilth, compaction, drainage, moisture, mineral content, temperature, and pH should be reported.

(D) Dimensions of test plots or sites and number of replicates should be reported. The type of experimental design used such as detailed description and diagram of the experimental test area should be reported.

(E) Number and length of crop rows, row spacing, and plant spacing within rows, if applicable, should be reported.

(F) If crops or crop sites are treated, a statement regarding cultivar name and other distinguishing characteristics (e.g., level of pest susceptibility) should be reported.

(G) When buildings are treated with pesticides, the number of rooms, their dimensions, and their spatial arrangements should be reported.

(viii) Geographic areas. Geographic areas (state, county, and town) where the tests were conducted, and the rationales for selection of these sites, should be reported.

(ix) Climatic factors. Critical environmental conditions at application time, such as precipitation, temperature, sunlight, humidity, and wind velocity, should be reported. Abnormal climatic conditions may occur within a given area which cannot be considered in the test design but these may markedly affect results. Such conditions and effects observed should be reported in the discussion or conclusions.

(x) Pest populations and crop stage. Target pest population levels at the beginning of treatment, at periodic intervals after treatment, and at the end of the test period should be reported when applicable. The growth stages of the pests and host plants should be reported. Crop growth stage should be referenced to the number of days before or after planting, emergence, or specific development stage or to its height. Whenever possible, the general level of the pest problem being tested should be characterized (light, moderate, severe, or similar phraseology).

(xi) Cultural practices. When applicable, information is needed on cultural practices that may affect pesticide application to crops because of their impact on product performance. Where applicable, the report must include information on seedbed preparation, seed planting depth, cultivation practices, and supplemental irrigation. Additional details on irrigation practices as an experimental variable are discussed in paragraph (e)(3)(v)(C) of this section.

(xii) Observation times. The interval between treatments and observations for pest control should be reported. Observations for efficacy and adverse effects should be made at intervals which indicate minimum response times and duration of effects. Dates observed and percent control of specific pests or plant responses to growth regulators, desiccants, or defoliants compared to untreated controls and to commercial pesticides (if used as standards) should be reported.

(xiii) Unusual events. Pertinent comments regarding effects test conditions on performance should be reported, particularly when they adversely affect the level of product performance or would invalidate the test data obtained.

(xiv) Mode of pesticide entry, movement, and action. A description of the mode of action and movement of the pesticide (e.g., translocation, tenacity, redistribution through rain) should be submitted or referenced when known. For a pesticide used to control vertebrate animals, the report should note how the pesticide enters the pest organism, such as by body contact, inhalation, oral ingestion, or by any combination of these routes.

(xv) Statistical procedures. A description of the statistical procedures used in the test design and analysis should be submitted.

(4) Performance evaluations. (i) A special section of the test report should be devoted to product performance evaluations. The following are examples of systems that may be used to evaluate the submitted data:

(A) A rating scale (or percent) showing performance related to efficacy and commercial acceptability as a rating score. Descriptive criteria for each numerical value if a rating scale should be presented.

(B) Dose-response data for all site/pest combinations for which registration is proposed.

(C) Clearly defined statements of benefits, such as increased yields, unblemished fruits, reduction in nuisance pest levels, reduced disease incidence, fewer rat bites, to be derived from the pesticide use. The applicant should indicate what he considers to be a commercially acceptable level of pest control.

(ii) Refer to Subdivision J for reporting phytotoxicity. Report other adverse effects such as spotting of paint, weakening of cloth or fibers, presence or odors of dead pest organisms, secondary poisoning, increase of nontarget species to intolerable levels, and similar adverse or undesirable results.

(f) Supporting statements. An applicant may submit written statements of opinion regarding the efficacy and limitations of a particular product, when expressed by individuals reasonably expert in observation and having experience with repeated use of such products. Evidence of the expert's experience should accompany such statements. Testimonials or letters of recommendations from individuals with less than the qualifications described in this paragraph are not acceptable as support for effectiveness claims.

§ 90-30 Acceptable methods.

Reference 1 is useful in developing test procedures on sensory methods of evaluation. These include test procedures on flavor, odor, taste, palatability, and repellency. [See paragraph (d)(2) of this section.]

References 2-5 are useful in developing sound test designs and employing statistical procedures for evaluations of product performance. [See paragraph (b)(8) of this section.]

1. Anonymous. 1968. Manual on Sensory Testing Methods. STP 434. Amer. Soc. for Testing & Materials, 1916 Race St., Philadelphia, Pa. 19103. 82 pp.

2. Cochran, W.G., and G.M. Cox. 1957. Experimental Designs, 2nd Ed. John Wiley and Sons, Inc.: N.Y. 611 pp.

3. LeClerg, E.L., W.H. Leonard, and A.G. Clark. 1966. Field Plot Technique. 2nd Ed. Burgess Publ. Co.: Minneapolis, Minn. 373 pp.

4. Snedecor, G.W., and W.C. Cochran. 1969. Statistical Methods. 6th Ed. Iowa State College Press: Ames, Iowa. 593 pp.

5. Steel, R.G.P., and J.H. Torrie. 1960. Principles and Procedures of Statistics. 2nd Ed. McGraw-Hill Book Co., Inc.: N.Y. 481 pp.

Series 91: EFFICACY OF ANTIMICROBIAL AGENTS

Subseries 91A: PUBLIC HEALTH USES

§ 91-1 General requirements.

(a) Scope. Section series 91-1 to 91-8 contains requirements for testing and performance of antimicrobial pesticide products with public health uses for which efficacy test data are required to be submitted to support registration. These include all antimicrobial products intended to control microorganisms infectious for man in any area of the inanimate environment where these microorganisms may present a hazard to human health. The label claims for an antimicrobial product determine whether or not it is considered to be related to human health. Refer to Subdivision H, §§ 101-1(b),(c),(d), and § 101-30 (Label Identification of Health-Related and Non-Health Related Claims for Antimicrobial Agents) for additional information on the relationship between label claims, human health considerations, and performance requirements for antimicrobial products. For those uses of antimicrobial pesticide products which are identified as not directly related to human health, guidance for testing and performance are provided in Subseries 91B: Non-Public Health Uses.

(b) General testing requirements. To fully comprehend the data required to demonstrate the effective performance of various types of antimicrobial pesticides, the applicant should understand the following basic information. This information is critical to the development and submission of appropriate data.

(1) Test substance. Unless otherwise specified, antimicrobial pesticides must be tested on the formulation to be offered for sale and, in some cases (e.g., pressurized sprays) with the product in the same packaging intended to be marketed. The manufacturer must also submit effectiveness data to show that he can consistently reproduce the formulation (batch replication), as well as to show that the product will retain its effectiveness for a minimal period of storage under average conditions to which it is likely to be exposed (shelf-life stability).

(2) Test methods. The product must be tested in accordance with the proposed directions for use. Section 91-30 contains citations for AOAC test methods referred to in this section. These methods, and supplemental modifications for registration testing, as well as other suggested test protocols or criteria, are entered separately in the § 91-30 or are described individually in the following sections.

(3) Neutralizers. In testing the efficacy of any antimicrobial product, appropriate neutralizers should be employed in the microbiological assay system, and evidence must be submitted to show that the neutralizer employed inactivates the active ingredient(s) and does not possess any

antimicrobial activity itself. In lieu of chemical neutralization, it must be documented that appropriate subculture techniques have been employed that preclude residual carryover of active ingredient(s). (See § 91-30(e) Supplemental recommendation No. 7.)

(4) Duration of testing. All products tested by methods referenced in the § 91-30 may be tested at the exposure periods prescribed in those methods. However, if the product is to be represented in labeling for use at exposure periods shorter or longer than those specified in the method, the method must be modified, in a manner acceptable to the Agency, to reflect the deviation in exposure intended. (See § 91-30(e) Supplemental recommendation No. 1. Refer also to Labeling Guidelines for Pesticide Use Directions, Subdivision H, §§ 101.)

(5) Reuse of product. Efficacy data must be developed to substantiate label directions and claims in regard to the number of days or the number of single applications a prepared use solution of an antimicrobial product, such as (but not limited to) surgical instrument sterilizers or surgical instrument disinfectants, can be used or reused before a fresh solution must be prepared. (Refer also to § 91-30 Supplemental recommentation No. 5 and Subdivision H §§ 101.) Such data must show retention of the claimed level(s) of antimicrobial activity in the use solution after repeated microbial and other appropriate challenges for the period of time or number of times specified in the directions for use.

(6) Variations in testing. The protocol for testing, test methods, and basic test elements will vary according to the type of product, type of substrate to be treated, proposed use pattern, label claims, directions for use, and other factors peculiar to that product. In many cases, specific requirements (including such elements as replication) can be provided only after consideration of all these factors. Before initiation of in-use or simulated-use studies, the proposed test protocols may be submitted for review and comment by the Agency. Complete details of all testing procedures and test/control results must be submitted. (Refer to § 91-30 for guidance.)

(7) Hard water claim. Any product that bears label claims for effectiveness in hard water must be tested by the appropriate method in synthetic hard water at the level claimed. [See § 91-30(e) Supplemental recommendation No. 3].

(8) Organic soils. Any product that bears label claims for effectiveness as a "one-step" cleaner-disinfectant, cleaner-sanitizer, or in the presence of light or moderate organic soils must be tested by the appropriate method in the presence of a representative organic soil, such as 5% blood serum. [Refer to § 91-30(e) Supplemental recommendation No. 4.] Additional organic material need not be incorporated into those procedures where at least 5% blood serum is already present in the inoculum to be dried on the surface. When a product is recommended for certain patterns of use where the organic soil claimed is of a specific type (such as soap film residue or hard water scum), the product must be tested in the presence of that specific organic soil.

(9) Validation of efficacy. The Agency reserves the option to perform its own tests for validation of efficacy of products selected on a case-by-case basis. If the applicant or registrant is notified by the Agency that samples are required for Agency testing, such samples shall be sent to EPA Microbiology Laboratory at the following address: Building 406, Agr. Res. Center - East, Beltsville, Md. 20705.

(10) Test failure. Failure of a product to meet the specified testing or performance requirements constitutes evidence that the product is unlikely to be effective as claimed in actual use. Refer to § 101-1(c) of Subdivision H for alternatives which can be considered in such cases.

(c) Terminology. Because of the variety of microorganisms to be controlled and the different claims and many use patterns of antimicrobial products, uniform label terminology and a common understanding of a few key words are important to a program for evaluating product performance. Therefore, the principal labeling terms used to describe antimicrobial activity and performance are defined in § 101-1(d) of Subdivision H.

§ 91-2 Products for use on hard surfaces.

(a) Sterilizers. The following apply to all products represented in labeling as sporicidal or sterilizing agents:

(1) Test standard. The AOAC Sporicidal Test (§ 91-30 Recommended method No. 1) is required. Sixty carriers representing each of two types of surfaces (porcelain penicylinders and surgical silk suture loops) are required to be tested against spores of both *Bacillus subtilis* (ATCC 19659) and *Clostridium sporogenes* (ATCC 3584) on 3 samples representing 3 different batches, one of which is at least 60 days old (240 carriers per sample, or a total of 720 carriers). Any sterilizing agent (liquid, vapor, or gas) which is recommended for use in a specific device must be tested by the AOAC Sporicidal Test in that specific device and according to the directions for use.

(2) Performance standard. Killing on all of the 720 carriers is required. Data submitted to support sterilizing claims will be subject to validation by tests conducted in the Agency's Microbiological Laboratory before the product submitted for registration will be considered acceptable.

(b) Disinfectants (limited efficacy). When a disinfectant is recommended in labeling for use against a specific major group of microorganisms only (such as Gram-negative or Gram-positive bacteria), it is considered to have only limited effectiveness, and consequently, only limited value as a disinfectant. The following requirements apply to such products:

(1) Test standard. Limited disinfectant activity must be substantiated with efficacy data derived from either the Association of Official Analytical Chemists (AOAC) Use-Dilution Method for water-soluble powders

and liquid products (§ 91-30 Recommended method No. 2), or the AOAC Germicidal Spray Products Test (§ 91-30 Recommended method No. 3) for spray products. Sixty carriers are required on each of 3 samples, representing 3 different batches, one of which is at least 60 days old, tested against Salmonella choleraesuis (ATCC 10708) for effectiveness against Gram-negative bacteria, or Staphylococcus aureus (ATCC 6538) for effectiveness against Gram-positive bacteria.

(2) Performance standard. The product must kill the test microorganisms on 59 out of each set of 60 carriers to provide significance at the 95% confidence level.

(c) Disinfectants (general or broad-spectrum efficacy). When a disinfectant is represented in labeling as having a broad spectrum of activity, more extensive testing is required. The following requirements apply to such products.

(1) Test standard. Sixty carriers on each of 3 samples, representing 3 different batches, one of which is at least 60 days old, must be tested against both S. choleraesuis and S. aureus. [Employ the AOAC Use-Dilution Method (§ 91-30 Recommended method No. 2) or the AOAC Germicidal Spray Products Test (§ 91-30 Recommended method No. 3).]

(2) Performance standard. Same as in paragraph (b)(2) of this section.

(d) Disinfectants (hospital or medical environment efficacy). (1) Test standard. The following apply when a disinfectant is recommended in labeling for use in hospitals, clinics, dental offices, or any other medical-related facility: Sixty carriers are required on each of 3 samples, representing different batches, one of which is at least 60 days old, tested against each of the following: S. aureus, S. choleraesuis, and Pseudomonas aeruginosa (ATCC 15442). [Employ the AOAC Use-Dilution Method (§ 91-30 Recommended method No. 2), or the AOAC Germicidal Spray Products Test (§ 91-30 Recommended method No. 3).]

(2) Performance standard. Same as in paragraph (b)(2) of this section.

(e) Fungicides (pathogenic fungi). These requirements apply to disinfectants which bear additional label claims of effectiveness against fungi pathogenic to man:

(1) Test standard. Effectiveness of liquid disinfectants against pathogenic fungi must be supported by efficacy data derived from each of 2 samples representing 2 different batches using the AOAC Fungicidal Test (§ 91-30 Recommended method No. 4) against Trichophyton mentagrophytes (ATCC 9533 is suitable).

(2) Performance standard. Killing of all fungal spores is required.

(3) Alternative test standard. Alternatively, the AOAC Use-Dilution Method, modified to conform with appropriate elements in the AOAC Fungicidal Test, may be employed. If the product is intended for use as a spray, the AOAC Germicidal Spray Products Test must be employed. Ten carriers on each of 2 samples representing 2 different batches must be employed in the test.

(4) Alternative performance standard. Killing of the fungal spores on all carriers in required.

(f) Virucides. These requirements apply to disinfectants which bear additional label claims of effectiveness against viruses. Most virucidal pesticides are intended for use on dry inanimate surfaces. For this reason, acceptable virological data are usually developed by carrier methods.

(1) Test standard. The test must simulate in-use conditions. The specific virus to be tested must be inoculated onto hard surfaces, allowed to dry, and is then treated with the product according to the directions for use on the product label. Each specific virus against which effectiveness is claimed must be treated. To demonstrate virucidal activity of a product against dried viruses on inanimate surfaces, petri dishes, glass slides, stainless steel cylinders, or other appropriate test surfaces may be used. The test surface must show a recoverable virus titer of at least 10^4 particles per surface. Virucidal performance must be demonstrated for each of two batches of product, each batch to employ a single surface specimen in the test. (Refer to § 91-30 Recommended method No. 5.)

(2) Performance standard. Inactivation of virus must be demonstrated at all dilutions when no cytotoxicity is observed in the assay system, or at all dilutions above the cytotoxic level when it is observed. The data must demonstrate at least a 3-log reduction in viral titer for both samples when cytotoxicity is present. The calculated viral titers must be reported with the test results.

(g) Tuberculocides. The following requirements apply to disinfectants which bear additional label claims of effectiveness as tuberculocides:

(1) Test requirements. Effectiveness against *Mycobacterium tuberculosis* must be substantiated with data derived on 10 carriers by the AOAC Tuberculocidal Activity Method [II. Confirmative In Vitro Test for Determining Tuberculocidal Activity (§ 91-30 Recommended method No. 6)] against *M. tuberculosis* var. *bovis* (BCG) for each of 2 samples representing two different batches of a liquid product under test. If the product is a spray, the procedure must be modified to conform with the AOAC Germicidal Spray Products Test using the media, test culture, and other elements described in the AOAC Tuberculocidal Activity Method.

(2) Performance standard. Killing of the test microorganisms on all carriers, and no growth in any of the inoculated tubes of two additional media, is required.

(h) Phenol coefficient(s). Data from this test are required only when permitted phenol coefficient claims are made in labeling of disinfectants. [See § 101-3 (k) of Subdivision H for those labeling claims which are permitted.]

(1) Test standard. Phenol coefficients for *Salmonella typhi* (ATCC 6539), the only official test organism, and for any additional Gram-negative or Grampositive asporogenous bacteria must be determined by the AOAC Phenol Coefficient Method (§ 91-30(e) Recommended method No.7) on each of two samples representing 2 different batches against each bacterium.

(2) Performance standard. The phenol coefficient is a numerical value that compares the bactericidal concentration of a disinfectant to the bactericidal concentration of pure phenol. This numerical value is obtained by dividing the greatest dilution of disinfectant killing *S. typhi* in ten minutes, but not in five minutes, by the greatest dilution of phenol showing the same results. Since phenol coefficient values are usually an unreliable index as to the effective use-dilution of a disinfectant product, the AOAC Use-Dilution Method must be employed to determine the actual disinfecting efficacy of a product, and its effective use dilution.

(i) Additional microorganisms. The following requirements apply to disinfectants which bear label claims against specific microorganisms other than those named in the AOAC Use-Dilution Method, AOAC Germicidal Spray Products Test, AOAC Fungicidal Test, and AOAC Tuberculocidal Activity Method (see § 91-30 Recommended methods Nos. 2, 3, 4 and 6), and not including viruses [see paragraph (f) of this section for virucides].

(1) Test standard. Effectiveness of disinfectants against additional specific microorganisms must be determined by either the AOAC Use-Dilution Method (§ 91-30 Recommended method No. 2) or the AOAC Germicidal Spray Products Test (§ 91-30 Recommended method No. 3), as appropriate, on 10 carriers for each of 2 samples representing 2 different batches against each microorganism.

(2) Performance standard. Killing of the test microorganisms on all carriers is required. Plate count data, on appropriate culture media, must be submitted on each test miroorganism to demonstrate that a concentration of at least 10^4 microorganisms survive the carrier-drying step to provide meaningful results at the 95% confidence level. [Refer to § 91-30(e) Supplemental recommendation No. 6.]

(j) Sanitizers (for non-food-contact surfaces). The following requirements apply to products bearing label claims for effectiveness as sanitizers for inanimate hard surfaces other than those which come in contact with food or beverages (e.g., floors, walls, furnishings).

(1) Test standard. These products must be tested by a protocol incorporating the basic elements outlined in § 91-30 Recommended method No. 8.

(2) Performance standard. The results must show a reduction of at least 99.9% in the number of each test microorganism over the parallel control count.

(k) Sanitizing rinses (for previously cleaned food-contact surfaces). The following requirements apply to any product with a label recommendation for treatment of previously-cleaned food-contact surfaces (e.g., eating and drinking utensils and food processing equipment) as a terminal sanitizing rinse. Antimicrobial agents applied to food-contact surfaces are defined as incidental food additives under the Federal Food, Drug, and Cosmetic Act, as amended (21 U.S.C. 201 et seq.), and require a food additive regulation or an exemption from such regulation. Recommendation of a potable water rinse after treatment does not preclude this requirement.

(1) Halide chemical products. Efficacy of sanitizing rinses formulated with iodophors, mixed halides, and chlorine-bearing chemicals must be demonstrated by data derived from the AOAC Available Chlorine Germicidal Equivalent Concentration Method (§ 91-30 Recommended method No. 9).

(i) Test standard. Data from one test on each of 3 samples, representing 3 different batches, one of which is at least 60 days old, against Salmonella typhi (ATCC 6539) are required.

(ii) Performance standard. Test results must show product concentrations equivalent in activity to 50, 100, and 200 ppm of available chlorine. (The reference standard in sodium hypochlorite.)

(2) Other chemical products. The efficacy of sanitizing rinses formulated with quaternary ammonimum compounds, chlorinated trisodium phosphate, and anionic detergent-acid formulations must be substanitated with data derived from the AOAC Germicidal and Detergent Sanitizers Method (§ 91-30 Recommended method No. 10).

(i) Test standard. Data from the test on one sample from each of 3 different batches, one of which is at least 60 days old, against both Escherichia coli (ATCC 11229) and Staphylococcus aureus (ATCC 6538) are required. When claims for the effectiveness of the product in hard water are made, all required data must be developed at the hard water level claimed.

(ii) Performance standard. Acceptable results must demonstrate a 99.999% reduction in the number of each test microorganism within 30 seconds. The results must be reported according to the actual count and percentage reduction over the control.

(l) Residual bacteriostatic activity of dried chemical residues on hard inanimate surfaces. Bacteriostatic claims are permitted only against microorganisms identified as causing economic or aesthetic problems (e.g., odor-causing bacteria) in the presence of moisture; but not for public health uses. Testing and performance guidance for non-public health uses are provided in Subseries 91B.

(m) Residual self-sanitizing activity of dried chemical residues on hard inanimate surfaces. The following requirements apply to products which bear label claims to provide residual self-sanitizing activity (i.e., significant reduction in numbers of infectious microorganisms which may be present or subsequently deposited) on treated surfaces that are likely to become and remain wet under normal conditions of use.

(1) Test standard. Each test must include the following basic elements:

(i) It must be based upon an adequately controlled in-use study or simulated in-use study employing as test microorganisms those target pathogens that are likely to be encountered in the environment in which the product is to be used.

(ii) Inocula of the test microorganisms at a sufficient concentration to provide at least 10^4 survivors on the parallel control surface must be employed for initial and subsequent challenges.

(iii) The residue on the treated surface(s) must be activated by the addition of moisture in a manner and over an exposure period identical to the use pattern for which the product is intended.

(iv) Quantitative bacteriological sampling must be conducted at frequent and regular intervals.

(v) The same type(s) of surface without the treatment must be employed in the test and inoculated in a manner and over an exposure period identical to the use pattern for which the product is intended.

(vi) The environmental conditions, such as relative humidity and temperature, employed in the test must also be reported; these must be the same as those which are likely to be encountered under normal conditions of use.

(5) Performance standard. For residual self-sanitizing claims, it must be demonstrated that at least 99.9% reduction in the numbers of test microorganisms occurred on the treated surface(s) over that of the parallel control surface(s).

§ 91-3 Products requiring confirmatory data.

(a) Specific situations. Confirmatory data (supplemental to the basic reference data) are required in certain situations in which an applicant intends to utilize previously submitted basic efficacy data to support an application or amendment for registration of a product. Confirmatory data are required on an applicant's own finished product to demonstrate his ability to produce an effective formulation. There are three commonly encountered situations in which an applicant is permitted to use previously submitted basic efficacy data, and submit only confirmatory data to support the registration of the antimicrobial product. These three specific situations and the corresponding required confirmatory data are described below. These specific confirmatory data are not applicable to any other categories of antimicrobial products. For use patterns other than those indicated below, required confirmatory data will be determined by the Agency on a case-by-case basis.

(b) Product formulations which are identical or diluted forms of a registered product. In this situation, the product proposed for registration has formulation, claims, and recommendations for use identical to those of a product already registered and manufactured by another registrant. The proposed product is merely repackaged, relabeled, or is a simple dilution.

(1) Data standard. A document substantiating that the product is formulated by the registrant for another applicant must be submitted. Specific references to the supporting data developed for the original product are also required.

(2) Test standard. If the product is a pressurized spray, all materials and devices must be shown to be identical to those utilized by the basic registrant. Furthermore, the filler packaging company must be the same for both products. If this identity cannot be substantiated, the applicant must submit complete efficacy data by the appropriate test method, which will be based upon the claims made for the product. [See paragraphs (b) thru (g) of § 91-2 of this Subdivision for test and performance standards.] Specific references to supporting data developed for the original product are also required.

(3) Performance standard. All documentation submitted must be appropriate and correct.

(c) Duplicated product formulations. In this situation, the formulation for the product proposed for registration duplicates a formulation which is registered, but the two products are not manufactured (or packaged) by the same company. The chemical composition, label claims, and recommendations for use are identical (in substance) to those accepted for the original registered product. See paragraphs (e) through (h) of this section for the test standards and performance standards for the confirmatory data that are to be submitted. Specific references to supporting data developed for the original product are also required.

(d) Minor formulation change in a currently registered product. In this situation, the change in the formulation is relatively minor, e.g., change in the level of an inert ingredient. The label claims and recommendations for use are identical to those accepted for the registered formulation. See paragraphs (e) through (h) of this section for the test standards and performance standards for the confirmatory data that are to be submitted. Specific references to supporting data developed for the original product are also required.

(e) Disinfectants (limited efficacy). (1) Confirmatory test standard. Ten carriers on each of two samples representing 2 different batches must be tested against either *Staphylococcus aureus* or *Salmonella choleraesuis*, depending upon the microorganism against which the activity of the product is limited. [See paragraph (b) in § 91-2 of this subdivision.] The AOAC Use-Dilution Method for liquids, or the AOAC Germicidal Spray Products Test for spray products, must be used with the same modifications employed for the original referenced data. Certification is required specifying that all parts and materials used in manufacturing the container for pressurized spray disinfectants are identical to those specified by the basic manufacturer.

(2) Confirmatory performance standard. Killing of the test microorganisms on all carriers is required.

(f) Disinfectants (general or broad spectrum efficacy). (1) Confirmatory test standard. Ten carriers on each of two samples representing two different batches must be tested against both *S. aureus* and *S. choleraesuis*, using the AOAC Use-Dilution Method for liquids, or the AOAC Germicidal Spray Products Test for spray products, are required with the same modifications employed for the original referenced data. Certification is required specifying that all parts and materials used in manufacturing the container for pressurized spray disinfectants are identical to those specified by the basic manufacturer.

(2) Confirmatory performance standard. Killing of the test microorganisms on all carriers is required.

(g) Disinfectants (hospital or medical environment efficacy). (1) Confirmatory test standard. Ten carriers on each of two samples representing 2 different batches are required against each of the following: *S. choleraesuis*, *S. aureus*, and *Pseudomonas aeruginosa*. The AOAC Use-Dilution Method for liquid products, or the AOAC Germicidal Spray Products Test for spray products, must be used with the same modifications employed for the original referenced data. Certification is required specifying that all parts and materials used in manufacturing the container for pressurized spray disinfectants are identical to those specified by the basic manufacturer.

(2) Confirmatory performance standard. Killing of the test microorganisms on all carriers is required.

(h) Sanitizers (for previously cleaned food-contact surfaces). (1) Confirmatory test standard. One test on one sample, with or without hard water (depending on label claims), is required using either: The AOAC Germicidal and Detergent Sanitizers Test (§ 91-30 Recommended method No. 10) against *Escherichia coli* for quaternary ammonium compounds, chlorinated trisodium phosphate, and anionic detergent-acid formulations; or the AOAC Available Chlorine Germicidal Equivalent Concentration Test (§ 91-30 Recommended method No. 9) against *Salmonella typhi* for iodophors, mixed halides, and chlorine-bearing chemicals.

(2) Confirmatory performance standard. See paragraphs (k)(1)(ii) and (k)(2)(ii) in § 91-2 of this subdivision for acceptable results.

§ 91-4 Products for use on fabrics and textiles.

(a) Laundry additives. The following requirements apply to antimicrobial products which bear label recommendations for use in the treatment of laundry (as a pre-soak treatment or in household and commercial laundry operations) to provide various levels of antimicrobial activity including disinfection, sanitization, or residual self-sanitization.

(1) Disinfecting pre-soak treatment. Effectiveness of products for "one-step" cleaning and disinfecting of hard surfaces (in the presence of moderate amounts of organic soil) may be extrapolated to disinfecting

of soiled fabrics by total immersion in the use solution prior to routine laundry operations. The requirements are as follows:

(i) Test standard. The AOAC Use Dilution Method (§ 91-30 Recommended method No. 2) modified to include organic soil (§ 91-30(e) Supplemental recommendation No. 4) must be employed in accordance with § 91-2(b)(1), (c)(1), or (d)(1).

(ii) Performance standard. Same as § 91-2(b)(2).

(2) Disinfecting laundry additives (non-residual). The following requirements apply to products which bear label claims as disinfectants for use in automatic or manual washing machine operations.

(i) Test standard. A suggested protocol, published by Petrocci and Clarke in the Journal of the Association of Official Analytical Chemists, is referenced in the § 91-30 Recommended method No. 11. That protocol is a simulated in-use study. However, an actual in-use study utilizing washing machines may be employed. The following basic elements must be incorporated in either study:

(A) The test microorganisms are Klebsiella pneumoniae (ATCC 4352) and Staphylococcus aureus (ATCC 6538). If the product is intended for use on hospital linens, it must be tested against the test microorganism Pseudomonas aeruginosa (ATCC 15442).

(B) Propagation of cultures, fabric-to-water ratios, test materials, water temperatures, exposure time, subculture media, and the basic procedures must be the same as those specified in the Petrocci and Clark protocol.

(C) Tests must be conducted with 3 samples representing 3 different product batches, one of which is at least 60 days old. Each sample must be tested with 9 fabric swatches against each of the test bacteria.

(D) The method employed must be designed to include testing of both the fabric and laundry water (5 ml from the automatic washer, or 0.5 ml from the simulated washing device) in individual widemouth jars containing subculture media and neutralizers. The laundry water-to-media volume ratio must not exceed 1:40.

(E) Growth or no-growth must be recorded after a 48-hour incubation period.

(ii) Performance standard. There must be no growth in the fabric subcultures and no growth in the subcultures from the laundry water with each test microorganism.

(3) Sanitizing laundry additives (non-residual). The following requirements apply to products which bear label claims as sanitizers for use in automatic or manual washing machine operations.

(i) Test standard. The same type of studies referred to in paragraph (a)(2)(i) of this section must be employed for evaluating the efficacy of laundry additives intended to sanitize laundry, with the following exceptions:

(A) Each of the product samples must be tested with 3 cloth swatches against each of the required test bacteria.

(B) Quantitative bacteriological assays must be conducted and the plate counts reported for the cloth swatches and laundry water.

(ii) Performance standard. At least 99.9% reduction in bacteria over the control count for both laundry water and fabric must be demonstrated against each test microorganism.

(4) Self-sanitizing laundry additives (residual). The following requirements apply to products which bear label claims to provide residual self-sanitizing activity (i.e., significant reduction in numbers of infectious microorganisms which may contaminate the items) on treated fabrics when used in automatic or manual washing machine operations (usually in the final rinse). Label claims for residual antimicrobial activity on laundered materials or articles can only be considered in those situations when such materials are likely to become and remain wet (for example, diapers, and bed linens of incontinent persons) under normal conditions of use and storage between launderings.

(i) Test standard. A suggested protocol published by Petrocci and Clarke (referenced in § 91-30 Recommended method No. 11) is acceptable for treating the fabric. The basic elements outlined in the protocol of the "Quantitative Procedure" of the American Association of Textile Chemists and Colorists (AATCC) Test Method 100-1974 (see § 91-30 Recommended method No. 12) employing Staphylococcus aureus (ATCC 6538) and Klebsiella pneumoniae (ATCC 4352) are acceptable for evaluating the residual antimicrobial activity. However, 3 samples representing 3 different product batches must be tested, and the following modifications to the method must be incorporated:

(A) Use a sufficient number of swatches placed exactly on top of each other so that they completely absorb 1 ml of inoculum which is prepared to contain at least 10^7 microorganisms/ml;

(B) The number of swatches used per jar must be reported;

(C) Incubation of the swatches must be at 20-21°C (68-70°F);

(D) Quantitative bacteriological assays should be performed at the following time intervals: 0, 30 min., 1-hr, 2-hr, 3-hr, 6-hr, and 24-hr. Consideration can be given to fewer or different time intervals, depending on the label claims, on a case-by-case basis.

(ii) Performance standard. For residual self-sanitizing claims against pathogenic microorganisms, the reduction of each test microorganism must be at least 99.9% over the "0-time" control and the parallel untreated inoculated control.

(5) Bacteriostatic laundry additives (residual). Bacteriostatic claims are permitted only against microorganisms identified as causing economic or aesthetic problems (e.g., odor-causing bacteria); but not for public health uses. Testing and performance guidance for non-public health uses are provided in Subseries 91B.

(b) Carpet sanitizers. The following requirements apply to products bearing label claims for effectiveness as carpet sanitizers.

(1) Test standard. Sanitizers for pre-cleaned carpeting must be tested by a protocol incorporating the basic elements of § 91-30 Recommended method No. 13. If the product is intended to be represented in labeling as a "one-step" cleaner-sanitizer, the method must be modified by including an appropriate soil with the bacterial inoculum. The following requirements must be met:

(i) Three product samples representing 3 separate batches, one of which is at least 60 days old, must be tested against *Staphylococcus aureus* (ATCC 6538) and *Enterobacter aerogenes* (ATCC 13048) with 2 different types of representative synthetic carpeting, such as acrylic and polypropylene tufted-loop types. If the product is intended for use in hospitals or medical institutions, it must also be tested against *Pseudomonas aeruginosa* (ATCC 15442). If the product is also intended for use on wool carpeting, an additional representative sample of wool carpet must be tested; otherwise, the label must bear a disclaimer for use on wool. All type carpet samples tested must be fully identified by the pile fiber, pile yarn weight of finished carpet, pile density, and tuft height. Adequate controls must demonstrate that bacteriostatic agents in the carpet pile or backing do not interfere with the tests results.

(ii) The amount of solution applied to the sample carpeting in the tests must be determined and extrapolated to obtain the amount of the use solution of product to be applied to carpeting (volume per unit area) as stated on the label.

(2) Performance standard. A 99.9% reduction of test bacteria over the scrubbed control count must be demonstrated.

(c) Mattresses and upholstered furniture. The use of gases or vapors is currently the only effective and practical means of treating mattresses, upholstered furniture, pillows, and similar objects to sterilize, disinfect, or sanitize against microorganisms. The following requirements apply to products bearing such label recommendations.

(1) Test standard. Simulated-use studies in which artificially-contaminated articles of this type are employed must be performed to demonstrate the level of effectiveness intended, as follows:

(i) Each test article must be inoculated throughout the entire article;

(ii) Samples must be taken or withdrawn randomly from the entire treated article and cultured for microbial growth;

(iii) An adequate control using a similar untreated article must be employed in such a study;

(iv) The test protocol, including such elements as replication, test microorganisms, etc., will vary with the level of effectiveness intended and the directions for use of the product, but the basic elements described above must be incorporated in any test protocol;

(v) A complete description of the test protocol employed must be submitted.

(2) Performance standard. (i) Same as § 91-2(a)(2) for sterilizing;

(ii) Same as § 91-2(b)(2), (c)(2), or (d)(2) for disinfecting;

(iii) Same as § 91-2(j)(2) for sanitizing.

(d) Impregnated self-sanitizing fabrics and textiles. The following requirements apply to products intended for treatment of fabrics and textile materials, usually during the manufacturing process, to provide durable residual self-sanitizing activity (i.e., significant reduction in numbers of infectious microorganisms which may be subsequently deposited on the finished item) in the presence of moisture or wet contamination.

(1) Test standard. The test standard is the same as in § 91-2(m)(1) of this subdivision, employing as test and control surfaces the treated and untreated fabric material or finished items, as appropriate.

(2) Performance standard. Same as in § 91-2(m)(2) of this subdivision.

(e) Impregnated bacteriostatic fabrics and textiles. Bacteriostatic claims are permitted only against microorgainsms identified as causing economic or aesthetic problems (e.g., odor-causing bacteria); but not for public health uses. Testing and performance guidance for non-public health uses are provided in Subseries 91B.

§ 91-5 Air sanitizers

(a) General. (1) These requirements apply to products bearing label claims for treatment of air to temporarily reduce the numbers of airborne microorganisms. There is considerable evidence that glycol vapors produce significant decreases in numbers of viable airborne bacteria under relatively wide conditions of relative humidity and temperature when properly and continuously dispensed by a vaporizing device so as to maintain suitable concentrations in the air of enclosed spaces.

(2) With dispensers for the intermittent treatment of air, such as pressurized aerosols, several investigators have shown that glycols (triethylene, dipropylene, or propylene glycol) at concentrations of 5% or more in such formulations will temporarily reduce numbers of airborne bacteria when adequate amounts are dispensed under relatively ideal conditions.

(3) For other types of products intended for the treatment of air, claims for reducing numbers of airborne microorganisms will be considered, providing supporting experimental data are submitted to justify such claims.

(b) Test standard. No standard method for evaluating air sanitizers has been adopted. Proposed testing protocols for studies of this kind may be submitted for review and evaluation by the Agency prior to initiation of the test.

(1) Glycol products. For products containing at least 5% glycols (triethylene, dipropylene, and/or propylene glycols), quantitative chemical determinations must be performed, using an air sampling device, to show the concentration of glycol vapor achieved with the product, used as directed, in an enclosed experimental room or chamber.

(2) Other products. For products other than those specified in paragraph (b)(1) of this section, quantitative microbiological assays must be performed, using an air sampling device, to show the level of reduction of viable microorganisms achieved with the product, used as directed, in an enclosed experimental room or chamber. The primary test bacteria are *Staphylococcus aureus* (ATCC 6538) and *Klebsiella pneumoniae* (ATCC 4352). If the product is intended for use in hospitals or medical environments, *Pseudomonas aeruginosa* (ATCC 15442) must also be tested.

(3) The methodology employed, such as spraying and sampling procedures, and the environmental conditions in the room or chamber, such as temperature, relative humidity, etc., must be reported. Raw data, as well as any statistical or graphical interpretation of the results, must be included in the reports.

(c) Performance standard. (1) Glycol products. The results must show that adequate glycol vapor concentrations (50% saturation or more) are achieved in the air of the test enclosure.

(2) Other products. The results must show a viable count reduction of at least 99.9% over the parallel untreated control, after correcting for settling rates, in the air of the test enclosure with each of the required test bacteria.

§ 91-6 Products for processing and industrial uses.

(a) Bacteriostatic preservatives. Currently, only non-public health claims are permitted for products to control microorganisms recognized as causing economic or aesthetic problems in processing and industrial uses. Testing and performance guidance for these non-public health uses are discussed in § 91-53 of this subdivision. Examples of these uses include:

(1) Deterioration of water-based paints, metalworking fluids, and other industrial products;

(2) Fouling of kerosene-based fuels (including jet aviation fuel), diesel fuels, and heating oils;

(3) Bacterial growth and spoilage in cane or beet sugar processing.

(b) (Reserved.)

§ 91-7 Products for control of microbial pests associated with human and animal wastes.

(a) Treatments for toilet bowl and urinal surfaces. The following requirements apply to products bearing label claims as disinfectants or sanitizers for toilet bowl and urinal surfaces.

(1) Disinfectants. (i) Test standard. Products recommended for disinfection of surfaces of toilet bowls and urinals must be tested by the AOAC Use-Dilution Method (for liquid products), or the AOAC Germicial Spray Products Test (for spray products), in accordance with the requirements outlined in § 91-2(b)(1),(c)(1) or (d)(1) of this subdivision. Note that the contained bowl water (approximately 96 fl. oz.) must be taken into consideration in determining the approprate use dilution for testing.

(ii) Performance standard. Same as in § 91-2(b)(2) of this subdivision.

(2) Sanitizers. (i) Test standard. Products recommended for sanitization of toilet bowl and urinal surfaces must be evaluated by an appropriate protocol similar to the sanitizer test for non-food contact surfaces specified in § 91-2(j) of this subdivision (§ 91-30 Recommended method No. 8). However, the test microorganisms must be the same as those outlined in § 91-2(b) and (c) of this subdivision in accordance with the areas of use referred to therein.

(ii) Performance standard. The results must show a reduction of at least 99.9% of each test microorganism over the parallel control count.

(b) Sanitizers for toilet and urinal bowl water. The following requirements apply to products bearing label claims as sanitizers for toilet and urinal bowl water.

(1) Test standard. The product must be tested by a simulated-use study incorporating all of the following basic elements:

(i) The product must be added to samples of the bowl water from three toilets or urinals at the use concentration, employing the recommended method of dispensing. Untreated control samples from the three toilets or urinals must also be included.

(ii) When the product is automatically "metered" or dispensed in some other fashion into the bowl water (or urinal trap), the consistent accuracy of the concentration dispensed and maintained must be documented.

(iii) Inocula containing representative pathogenic Gram-positive and Gram-negative test bacteria must be added to the treated and control samples of the bowl water from each of the toilets or urinals to provide a concentration of at least 10^4 colony-forming units per ml.

(iv) Microbial counts of the treated bowl water and the control bowl water must be conducted at a minimum of three exposure intervals, in addition to a "0-time" control.

(2) Performance standard. The reduction of each test microorganism must be at least 99.9% over the "0-time" control and the parallel untreated inoculated control.

(c) Bacteriostatic treatments for self-contained toilet systems, vomitus absorbents, and bird and animal cage litter. Bacteriostatic claims are permitted for such uses against microorganisms identified as causing economic or aesthetic problems (e.g., odor-causing bacteria). Testing and performance guidance for non-public health uses are provided in Subseries 91B.

§ 91-8 Products for treating water systems.

(a) Drinking water for humans. (1) Public water supplies. Municipal drinking water must meet the requirements of the Safe Drinking Water Act (Public Law 93-523).

(i) Test standard. Evidence must be submitted that the chemical intended for use as a drinking water disintectant has been tested under the auspices of the Office of Drinking Water (WH-550) of the Agency.

(ii) Performance standard. Documentation must be submitted that the chemical tested as above has been accepted for use as a drinking water disinfectant under the auspices of the Office of Drinking Water of the Agency.

(2) Emergency water supplies. The following requirements apply to chemical additives such as solutions, powders, or tablets intended for emergency disinfection of small quantities of drinking water of questionable potability by the general public in the absence of bacteriological monitoring facilities:

(i) Test standard. Controlled simulated-use studies which represent actual use conditions must include the following basic elements:

(A) Representative levels of organic and inorganic soil contamination;

(B) Various water temperatures;

(C) The specific dosage and exposure period recommended for the proposed product;

(D) A variety of representative test microorganisms, including cysts, bacteria and viruses; and

(E) Quantitative determination of the level of microbial contamination of the water before and after treatment.

(ii) Performance standard. The treatment must eliminate all test microorganisms from the water.

(3) Water treatment units. (i) Water purifier units. Any unit intended for the treatment of raw water to eliminate the potential health hazard posed by microorganisms is identified as a water purifier. The unit may rely on physical filtration (pesticidal device), or chemical treatment (pesticide), or a combination thereof, to achieve the intended purpose of purifying microbiologically non-potable water by eliminating water-borne pathogens in the water itself. Those units, such as submicron membranes and absolute filters, which rely solely on a physical means of removal of microorganisms from water, are identified under the Act as devices, and are subject to regulation but not registration. The test requirements indicated below are for the units containing an antimicrobial chemical (pesticide).

(A) Test standard. Controlled in-use or simulated-use studies for the water purifier unit must be conducted under conditions representing its actual use employing a defined actual or simulated raw water source containing a high level of microbiological pollution, including waterborne cysts, bacteria, and viruses. The test design will vary with different types of units and patterns of use, but must include such basic elements as:

(1) Representative levels of organic and inorganic soil contamination;

(2) Various water temperatures;

(3) Documentation of the antimicrobial concentration found in the test system;

(4) Quantitative determinations of the microbial contamination level of the water before and after passage through the unit;

(5) Documentation of the duration of effectiveness or effective capacity of the unit before a replacement is necessary.

(B) Performance standard. The treatment must eliminate the microbial pollution from the water.

(ii) Bacteriostatic potable water treatment units. Only bacteriostatic claims are permitted for such units against microorganisms identifed as causing aesthetic problems (e.g., objectionable tastes, odors, and the like). Testing and performance guidance for non-public health uses are provided in Subseries 91B.

(b) Drinking water for poultry and livestock. Non-drug claims for treatment of poultry and livestock drinking water with antimicrobials to provide disinfection, sanitization, or bacteriostasis are not considered to be directly related to human health. However, such products require establishment of a tolerance from EPA under the Federal Food, Drug, and Cosmetic Act. Efficacy testing and performance guidance on non-public health uses are provided in Subseries 91B.

(c) Swimming pool water. The following requirements apply to products bearing label claims for swimming pool water disinfection. Numerous factors influence the concentrations necessary for disinfection of swimming pool water in practical applications: number of swimmers in the pool; frequency of use; frequency with which water is changed; general weather conditions; and types and degree of organic contamination of the water by the swimmers themselves (e.g., suntan lotions and oils) and by various debris. Therefore, a two-phased study (presumptive laboratory testing and confirmatory field testing) is required.

(1) Test standard. (i) Laboratory test. Presumptive efficacy of swimming pool water disinfectants may be indicated with data derived from the AOAC Method for Water Disinfectants for Swimming Pools (§ 91-30 Recommended method No. 14), or with slight modifications (e.g., pH) thereof, against both *Escherichia coli* (ATCC 11229) and *Streptococcus faecalis* (PRD).

(ii) Field test. In addition to the laboratory test requirements referred to in paragraph (c)(1)(i) of this section, confirmatory efficacy data shall be derived from in-use tests under an Experimental Use Permit in at least two swimming pools. The tests must be conducted for an entire swimming season (4 to 12 months). Reports must include (but are not limited to) the following information concerning the test pools:

(A) The daily bather load.

(B) The design of the pool, the recirculation and filter system, and water capacity.

(C) The amount and identification of all chemicals added daily to the swimming pool water (including the time, site, and method).

(D) The range of chemical characteristics of the swimming pool water, such as: pH, nitrogenous substances, metals, and hardness.

(E) The physical characteristics of the swimming pool water, including temperature and clarity, determined at least daily.

(F) Meteorological data, including air temperature, rainfall and number of hours of sunlight (determined daily) for outdoor pools.

(G) Bacteriological monitoring, conducted daily, in accordance with the Suggested Ordinance and Regulations Covering Public Swimming Pools of the American Public Health Association. (See reference in § 91-30 Recommended method No. 14).

(H) The concentration of the antimicrobial agent in the swimming pool water monitored daily at the same time-intervals that the bacteriological assay samples are obtained.

(I) The method that the product user will employ for monitoring the level (concentration in ppm) of antimicrobial agent contained in the pool water.

(2) Performance standard. (i) Laboratory test. The lowest concentration of the test germicide providing results equivalent to those of the sodium hypochlorite control is the lowest concentration of the product that can be considered effective.

(ii) Field test. The product must meet all of the efficacy requirements outlined in Suggested Ordinance and Regulations Covering Public Swimming Pools of the American Public Health Association.

(d) Control of Legionnaires' disease bacteria in industrial water systems. (Reserved)

§ 91-30 Acceptable methods.

(a) Scope. This section provides recommended methods for satisfying most efficacy data requirements for public health uses of antimicrobial agents. It also provides supplemental recommendations for modifying or expanding the recommended methods to obtain additional data necessary to support certain specific claims and/or special patterns of use.

(b) General. Depending upon the type of antimicrobial agent, target microorganism, and site to be treated, all tests must address those factors that would normally be expected to be encountered in the use pattern intended for the product, such as: the method of application; the nature of the surface, item, or substrate to be treated; the presence or absence of soil or other interfering conditions; temperature; exposure period; and the number of times or duration of time that the use solution can be used or re-used. The actual test procedure to be employed will vary according to the characteristics of the product, the target pest(s) and the pattern of use intended. Specification of methods in these Guidelines for all conceivable public health uses is not feasible, and the applicant must be responsible for the validity of the test method selected to substantiate efficacy. The applicants should assure themselves that the selected method is current and applicable to the product and use(s) proposed for registration. Reference to Labeling Guideines for Pesticide Use Directions, Subdivision H, should be made concurrently with consideration of product performance of antimicrobial agents.

(c) Reporting of data. Systematic and complete descriptions of the tests employed and results obtained are essential for proper review and evaluation of product performance by the Agency. All test reports must include identification of the testing laboratory or organization, when and where the tests were conducted, and the name of the person(s) responsible for the conduct of the tests.

(1) Recommended methods. When the recommended methods (such as standard AOAC tests) are employed to develop efficacy data, certain minimal information must be provided in the test report. The report must include, but is not limited to, the following:

(i) Test method employed, and any modifications thereto;

(ii) Test microorganisms employed, including identification of the specific strain (ATCC or other);

(iii) Concentration or dilution of product tested and how prepared;

(iv) Number of samples, batches, and replicates tested;

(v) Preparation date of each product batch (individually formulated preparation of the product);

(vi) Phenol resistance of test microorganisms (actual test results) when specified in the methods;

(vii) Identification of all material or procedural options employed, where such choice is permitted or recommended in the test method selected (for example, growth media, drying time for inoculated carriers, neutralizer and/or subculture media, secondary subculturing);

(viii) Complete report of results obtained for each individual replication;

(ix) Any control data essential to establish the validity of the test.

(2) Modification of recommended methods. Where recommended methods are significantly modified to support specific claims and/or use for a product, the protocol employed for modifying the test must be provided in specific detail with the test report. The applicant may submit the proposed modification for review and evaluation prior to initiation of the test.

(3) Other methods. When recommended methods, or modifications thereto, are not employed to develop efficacy data (such as actual inuse or many kinds of simulated-use testing), complete testing protocols must be submitted with the test reports. All materials and procedures employed in testing must be described in a manner consistent with original research reports published in technical or scientific journals. Where references to published reports or papers are made, copies or reprints of such references should be provided with the test reports. Proposed testing protocols for in-use or simulated-use studies of this kind may be submitted for review and evaluation by the Agency prior to initiation of tests.

(d) Recommended methods. (See the Supplemental recommendations for modifications appropriate for the intended pattern of use.)

(1) Sterilizers.

Horowitz, William, ed. Sporicidal test - official final action. Official Methods of Analysis of the Association of Official Analytical Chemists. Current Edition. Association of Official Analytical Chemists, Washington, D.C.

Also see: Beloian, A. 1978. Report on disinfectants. J. AOAC 61:372.

(2) Disinfectants water soluble powders and liquid products (Hard surfaces).

Horowitz, William, ed. Use-dilution method - official final action. Official Methods of Analysis of the Association of Official Analytical Chemists. Current Edition. Association of Official Analytical Chemists, Washington, D. C.

(3) Disinfectants - Spray products (Hard surfaces).

Horowitz, William, ed. Germicidal spray products - official final action. Official Methods of Analysis of the Association of Official Analytical Chemists. Current Edition. Association of Official Analytical Chemists, Washington, D.C.

(4) Disinfectants - Efficacy against pathogenic fungi (Hard surfaces).

Horowitz, William, ed. Fungicidal test - official final action. Official Methods of Analysis of the Association of Official Analytical Chemists. Current Edition. Association of Official Analytical Chemists, Washington, D.C.

(5) Disinfectants - Efficacy against viruses (Hard surfaces).

(Proposed method prepared by Registration Division, Office of Pesticide Programs, EPA, 1976)

The Agency will accept adequate data developed by any virological technique which is recognized as technically sound, and simulates, to the extent possible in the laboratory, the conditions under which the product is intended for use. For virucides whose use-directions identify the pesticide as one intended for use upon dry, inanimate, environmental surfaces (such as floors, tables, clean and dried medical instruments, etc.), carrier methods, which are modifications of either the AOAC Use Dilution Method (for liquid surface disinfectants) or the AOAC Germicidal Spray Products Test (for surface spray disinfectants), must be used in the development of the virological data. To simulate in-use conditions, the specific virus to be tested must be inoculated onto hard surfaces, allowed to dry, and then be treated with the product according to the directions for use on the product label. If the product is intended to be represented as virucidal in the presence of organic soil ("one-step"), an appropriate organic soil, such as 5% blood serum, must be included with the inoculum. Additional organic material need not be incorporated into those procedures where at least 5% blood serum is already present in the virus suspension used as the inoculum. The product must be tested against a recoverable virus titer of at least 10^4 from the test surface (such as petri dish, glass slide, cylinder) for a 10-minute exposure period at room temperature. The virus is then assayed by an appropriate virological technique. The protocol for viral assay must provide the following information:

(i) The virus recovered from a minimum of 4 determinations per each dilution in the assay system (tissue culture, embryonated egg, animal infection, or whatever assay system is employed).

(ii) The activity of the germicide against the test virus from a minimum of 4 determinations per each dilution in the assay system.

(iii) Cytotoxicity controls: the effect of the germicide on the assay system for a minimum of 4 determinations per each dilution.

(iv) Any special methods which were used to increase the virus titer and to detoxify the residual germicide.

(v) The ID-50 values calculated for each assay.

(vi) The test results shall be reported as the reduction of the virus titer by the activity of the germicide (ID-50 of the control less the ID-50 of the test system), expressed as log 10, and calculated by a statistical method (Reed and Muench, 1938; Litchfield and Wilcoxon, 1949; as examples).

(vii) For virucidal data to be acceptable, the product must demonstrate complete inactivation of the virus at all dilutions. When cytotoxicity is observed in the assay system (as in Tables 1, 2, and 3, below), at least a 3-log reduction in titer assay system must be demonstrated. The calculated viral titers must be reported with the test results. A typical laboratory report of a single test with one virus (recovered from a treated surface) involving a tissue culture, therefore, would include the details shown in the following tables:

Table 1. EXAMPLE OF HYPOTHETICAL TEST RESULTS DEMONSTRATING VIRUCIDAL ACTIVITY

Dilution - inoculated	Virus - Disinfectant[1]	Virus - Control[1]	Cytotoxicity - Control
10^{-1}	TTTT	++++	TTTT
10^{-1}	TTTT	++++	TTTT
10^{-2}	TTTT	++++	TTTT
10^{-3}	TOOO	++++	TOOO
10^{-4}	OOOO	++++	OOOO
10^{-5}	OOOO	++++	OOOO
10^{-6}	OOOO	+++O	OOOO
10^{-7}	OOOO	OOOO	OOOO
10^{-8}	OOOO	OOOO	OOOO

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[1] Recovery of virus from surfaces demonstrated by cytopathogenic effect, fluorescent antibody, plaque count, animal response, or other recognized acceptable technique.

Note: T = toxic; + = virus recovered; O = no virus recovered.

Table 2. CALCULATION OF THE TISSUE CULTURE INFECTIVE DOSE 50% ($TCID_{50}$*)

Dilution inoculated	No. infected/ No. inoculated	No. infected	No. not infected	Accumulated Values			
				No. infected	No. not infected	No. infected/ no. inoculated	Percent infected
10^{-1}	4/4	4	0	24	0	24/24	100
10^{-2}	4/4	4	0	20	0	20/20	100
10^{-3}	4/4	4	0	16	0	16/16	100
10^{-4}	4/4	4	0	12	0	12/12	100
10^{-5}	4/4	4	0	8	0	8/8	100
10^{-6}	3/4	3	1	4	1	4/5	80
10^{-7}	1/4	1	3	1	4	1/5	20
10^{-8}	0/4	0	4	0	8	0/8	0

*$TCID_{50} = 10^{6.5}$

Table 3. CALCULATIONS OF THE TISSUE CULTURE LETHAL DOSE 50% (TCLD$_{50}$*)

Dilution inoculated	No. toxic/ No. inoculated	No. toxic	No. not toxic	Accumulated Values			Percent toxic
				No. toxic	No. not toxic	No. toxic/ No. inoculated	
10^{-1}	4/4	4	0	9	0	9/9	100
10^{-2}	4/4	4	0	5	0	5/5	100
10^{-3}	1/4	1	3	1	3	1/4	25
10^{-4}	0/4	0	4	0	7	0/7	0
10^{-5}	0/4	0	4	0	11	0/11	0
10^{-6}	0/4	0	4	0	15	0/15	0
10^{-7}	0/4	0	4	0	19	0/19	0
10^{-8}	0/4	0	4	0	23	0/23	0

*TCLD$_{50}$ = $10^{2.7}$; therefore, virus inactivation = TCID$_{50}$ - TCLD$_{50}$ = $10^{3.8}$ log 10. Claims for virucidal activity for a product must be restricted to those viruses which have actually been tested.

(viii) Also see:

Litchfield, J.T., Jr., and F. Wilcoxon. 1949. A simplified method of evaluating dose-effect experiments. J. Pharm. Exp. Therapy 96:99-115.

Reed, L.J., and H. Muench. 1938. A simple method of estimating 50% endpoints. Amer. J. Hygiene 27:493-497.

(6) Disinfectants - Efficacy against Mycobacterium tuberculosis (Hard surfaces).

Horowitz, William, ed. Tuberculocidal activity - official final action. II. Confirmative In-Vitro Test for Determining Tuberculocidal Activity. Official Methods of Analysis of the Association of Official Analytical Chemists. Current Edition. Association of Official Analytical Chemists, Washington, D.C.

(7) Disinfectants - Phenol coefficients.

Horowitz, William, ed. Phenol coefficient - official final action. Official Methods of Analysis of the Association of Official Analytical Chemists. Current Edition. Association of Official Analytical Chemists, Washington, D.C.

(8) Sanitizers - Non-food contact surfaces.

(Proposed method prepared by Registration Division, Office of Pesticide Programs, EPA, 1976).

To substantiate the sanitizing claims for a product, the applicant must submit data to show that the product, when used as directed, will substantially reduce the numbers of test microorganisms on a treated surface over those on an untreated control surface. The following protocol may be utilized:

(i) The product must be tested against each test bacterium on each representative surface depending on the uses proposed on the label. The test microorganisms are *Staphylococcus aureus* (ATCC 6538) and *Klebsiella pneumoniae*, aberrant, (ATCC 4352). *Enterobacter aerogenes* (ATCC 13048) may be substituted for *K. pneumoniae*. Representative test surfaces include, but are not limited to: glass, metal, unglazed or glazed ceramic tile, or vitreous china. The propagation of cultures and the use of subculture media and other related equipment shall be as specified in Sec. 4.001 and 4.002 of the Official Methods of Analysis of AOAC, 12th ed. (1975).

(ii) Determine the count of bacteria in an 18- to 24-hr broth culture and add a 0.01- to 0.03-ml quantity of the broth culture by spreading on 1 x 1 in. square of test surface using a bacteriological loop. If the product is intended to be represented as a "one-step" cleaner-sanitizer, an appropriate organic soil, such as 5% blood serum, must be included with the inoculum. The square shall be dried for 20-30 min. in a bacteriological incubator at 30 to 37° C. A "zero time" bacterial numbers recovery test must be performed to show the efficiency of the recovery process and must be reported. The "zero time" test shall show the loss in viability that occurred during the drying. Apply the product to the test surface as directed on the label. Run parallel tests on the formulation with active ingredient(s) omitted in an identical manner to serve as the controls. If such a control solution is not suitable, use sterile distilled water containing 0.01% isooctylphenoxypolyethoxyethanol (9-10 moles oxyethylene, e.g., Triton X-100). After a suitable time interval, recover test organisms by washing the squares with adequate agitation in appropriate media or dilution fluid containing appropriate neutralizers. Make plate counts on appropriate nutrient agar containing the same neutralizers by the pour or spread plate technique. Exposure time intervals between zero time and 5 min. must be tested for the product as well as for the untreated controls.

(iii) The results must show a bacterial reduction of at least 99.9% over the parallel control within 5 min.

(iv) Also see:

Horowitz, William, ed. Official Methods of Analysis of the Association of Official Analytical Chemists. Current Edition. Association of Official Analytical Chemists, Washington, D.C.

(9) Sanitizing rinses - Food contact surfaces (Halides).

Horowitz, William, ed. Available chlorine germicidal equivalent concentration - official final action. Official Methods of Analysis of the Association of Official Analytical Chemists. Current Edition. Association of Official Analytical Chemists, Washington, D.C.

(10) Sanitizing rinses - Food contact surfaces (Non-halides).

Horowitz, William, ed. Germicidal and detergent sanitizers official final action. Official Methods of Analysis of the Association of Official Analytical Chemists. Current Edition. Association of Official Analytical Chemists, Washington, D.C.

(11) Disinfectants and sanitizers - Laundry additives.

Petrocci, A.M., and Paul Clark. 1969. Proposed test method for antimicrobial laundry additives. J. AOAC 52:836-842.

(12) Residual self-sanitizers - Laundry additives.

AATCC Committee RA31. 1974. AATCC Test Method 100-1974 (9. Quantitative Procedure) (Reference or Confirmatory Test). Pp. 264-265 in Technical Manual of the American Association of Textile Chemists and Colorists. Volume 50. American Association of Textile Chemists and Colorists, Research Triangle Park, N.C.

(13) Sanitizers - Carpets.

(Proposed method prepared by Registration Division, Office of Pesticide Programs, EPA, 1976; revised 1981)

(i) Special equipment and materials. (A) Carpet mounting board. Mount a piece of 1/8-in. (0.3 cm) tempered hardboard, tempered surface up, on a 16 x 16-in. (40.6 x 40.6 cm) base of 3/4-in. (1.8 cm) thick marine plywood, with 3/4-in. (1.8 cm) brads.

(B) Cutting equipment. 2 x 2-in. (5.1 x 5.1 cm) squares of 1/4-in. (0.6 cm) acrylic plastic with 3/32-in. (0.24 cm) holes in the center as templates, and a sharp knife with replaceable blade.

(C) Scrub brushes. 1 1/4 x 3 1/2-in. (4.2 x 8.9 cm) surgical hand brush with 5/8-in. (0.6 cm) nylon bristles.

(D) Extraction bottles. 8-oz. (236.6 ml), widemouth, round, polypropylene bottles with screw caps (Nalgene 2105 or equivalent) containing 10 stainless steel penicylinders and 100 ml of appropriate neutralizer broth. Similar style glass bottles may be used, but care must be taken to prevent breakage during shaking.

(E) Spray device. Adjustable spray atomizer modified to feed from a calibrated test tube or bottle. A Model 15 DeVilbiss atomizer on a 2-oz. (59.2-ml) bottle graduated with 10-ml marks may be used.

(F) Carpet. If the product is intended for use on commercial grade carpeting, two representative carpets, such as acrylic and polypropylene tufted-loop type must be tested. No carpeting is available to serve as a standard. If the product is intended for use on wool carpeting, a representative wool sample must additionally be tested. All carpet samples tested must be fully identified, and the pile fiber type, pile yarn weight of finished carpet, pile density and tuft height must be reported. Adequate controls must demonstrate that bacteriostatic agents in the carpet pile or backing do not interfere with the test results.

(ii) Test cultures and media. (A) Test bacteria. Use Staphylococcus aureus (ATCC 6538) and Enterobacter aerogenes (ATCC 13048). If the product is intended for use in hospitals, Pseudomonas aeruginosa PRD-10 (ATCC 15442) must additionally be tested.

(B) Nutrient Agar B. AOAC Methods, sec. 4.023 (a)(2).

(C) Phosphate buffer dilution water. AOAC Methods, sec. 4.023 (f).

(D) Double strength neutralizer broth. For phenolic based products, Letheen broth [AOAC Methods, sec. 4.001 (d)(3)] plus an additional 0.7 g lecithin (Azolectin) and 5 g polysorbate 80 (Tween 80) per liter may be used; or a defoaming neutralizer consisting of nutrient broth [AOAC methods, sec. 4.001 (a)] plus 1.0% Pluronic 25R2 (Meroxapol 252) has been suggested. In the case of halogen or heavy metal based products, 0.1% sodium thioglycolate and 0.01% isooctylphenoxypolyethoxyethanol (Triton X-100) in phosphate buffer (pH 7.2) may be used.

(E) Neutralizer plate count agar. Tryptone glucose extract agar [AOAC Methods, sec. 4.037 (a)] plus 0.7 g lecithin (Azolectin) and 5 g polysorbate 80 (Tween 80) per liter.

(iii) Bacterial inoculum. Prepare French square culture bottles with nutrient agar B and test bacteria (AOAC Methods, Sec. 4.026). Prepare standardized bacterial stock suspensions by washing growth from bottles and adjust to a density of 10 x 10^9 bacteria per ml with phosphate buffer dilution water (AOAC Methods, sec. 4.026).

(iv) Procedure. (A) Cut the carpet into 8 x 12-in (20.3 x 30.5 cm) pieces. With the aid of the 2 x 2-in. (5.1 x 5.1 cm) template, cut six 2 x 2-in. squares (2 rows of 3 squares per row) from the backing side of the carpet, leaving at least 4 in. (10.2 cm) between the center of each square. The preferred method is to leave about 1/8 in. (0.32 cm) of backing intact at each corner of each cut square so that the entire piece of carpeting can be sterilized and inoculated without separation. Mark the pile surface in the center of each test square with a waterproof marking pen with the aid of the hole in the center of the template. Cover the pile surface of the carpeting with aluminum foil and fold over edges to secure. Steam sterilize and dry. Only carpet that has been determined to be free from residual bacteriostatic activity on the pile or backing, following autoclaving, shall be used. A seeded agar plate overlay technique should be used for this determination.

(B) Dilute the standardized bacterial stock suspensions, prepared as in paragraph (d)(13)(iii) of this section, with phosphate buffer dilution water containing 0.01% isooctylphenoxypolyethoxyethanol to a concentration 10 x 10^7 bacteria per ml. Inoculate the previously marked center of each cut square with 0.1 ml of the bacterial suspension. (Retain the bacterial suspensions for determination of inoculation numbers). Dry inoculated carpet in an incubator at 35-37°C for 60 min. with the foil wrap loosely in place.

(C) Condition brushes by immersing the bristles in separate containers (15- cm glass petri dishes or equivalent) of diluted test solution and a control solution without the active antimicrobial ingredient(s) for 15 min. (If such a control solution is not available, use sterile distilled water containing 0.01% isooctylphenoxypolyethoxyethanol). Fasten 2 pieces of innoculated carpet (each containing 6 test squares) onto the carpet mounting board by nailing each corner with upholstery tacks, and with the foil wrapping positioned so as to protect the controls during spraying and scrubbing with the test solution. Place the board in a biological hood or glove box. A simple safety chamber can be constructed from a large plastic bag.

(D) Determine the amount of test solution intended to be applied to one piece of the carpeting containing 6 spots of dried bacterial inoculum [96 sq. in. or 2/3 sq. ft. (244 sq. cm.)] and subtract approximately 15 ml which will be applied later in the brushing procedure. Apply the predetermined amount of diluted test solution at room temperature uniformly by metered spray to one piece of the test carpet. Shake excess test solution from a conditioned brush and transfer to a fresh dish containing 100 ml of test solution at room temperature. Dip bristles of brush and transfer the retained test solution to an inoculated spot on the sprayed carpet.

Scrub the spot for 30 sec. using 30 circular clockwise strokes and 30 circular counterclockwise strokes. A circular area of pile approximately 3 in. (7.6 cm) in diameter around each spot must be covered by this treatment. Moderate to heavy pressure should be applied downward on the brush to work the solution to the base of the pile. Repeat dipping of brush into test solution and scrubbing procedure until each of the 6 spots is treated. The brush dipped into the solution no more than 6 times will deliver about 15 ml of solution to the carpet. Do not exceed this amount. Record the total volume of solution applied by spray and brush. Allow the treated carpet piece to remain at room temperature for 60 min. for partial drying of the treated areas.

(E) While the piece of carpet treated with the test solution is drying, spray the non-active control solution at room temperature onto half of the other (control) piece of carpet so as to cover 3 of the 6 spots of dried inoculum. Position the aluminum foil over the remainder. Spray an amount equivalent to half of the amount of sprayed test solution. Scrub the 3 wet spots in the same manner as the test carpet. The remaining 3 spots are unscrubbed controls to determine the numbers of bacteria which survived drying of the inoculum. Care must be taken not to wet or scrub over the unscrubbed control area. Allow the scrubbed and unscrubbed controls to remain at room temperature for 60 min. as with the test piece.

(F) Following the 60-min. drying periods, cut each 2 x 2-in. test square free with flamed forceps and knife. Transfer each square of carpet to a separate extraction bottle of neutralizer broth. Shake each extraction bottle vigorously for at least 1 min. to free the bacteria from the carpet fibers. Determine the number of viable bacteria in each sample bottle by plating duplicate dilutions in neutralizer place count agar. Similarly determine the number of viable bacteria in 0.1 ml of the suspension used for inoculating the carpet. Also incubate all broth extraction bottles to determine whether neutralization of the test sample was achieved.

(G) Determine the percent reduction of viable bacteria by the test solution by comparing the number of survivors from each treated test square against the average viable count from the scrubbed control squares. An average viable count of at least 1.0×10^6 bacteria from the extracted unscrubbed control squares is necessary for a valid test.

(v) Also see:

Horowitz, William, ed. Official Methods of Analysis of the Association of Official Analytical Chemists. Current Edition. Association of Official Analytical Chemists, Washington, D.C.

(14) Disinfectants - Swimming pools.

Horowitz, William, ed. Water disinfectants for swimming pools official final action. Official Methods of Analysis of the Association of Official Analytical Chemists. Current Edition. Association of Official Analytical Chemists, Washington, D.C.

(i) Also see:

Joint Committee on Swimming Pools of the A.P.H.A. in cooperation with the U.S.P.H.S. 1964. Suggested Ordinance and Regulations Covering Public Swimming Pools. The American Public Health Association, New York, N.Y.

(e) Supplemental recommendations. When an antimicrobial agent is intended for a use pattern that is not reflected by the test conditions specified in the recommended methods indicated above, one or more test conditions specified in the method must be modified and/or supplementary data developed in order to provide meaningful results relative to the conditions of use. The following information is critical to the development and submission of appropriate data.

(1) Exposure period. The exposure period required for an antimicrobial agent to be effective may be shorter or longer than the exposure period specified in the recommended method. A modification to provide a shorter exposure period is restricted by the manipulative limitations inherent in the method, while a modification to provide a longer exposure period is restricted by the practical considerations of the use pattern.

(2) Type of surface. When an antimicrobial agent is intended to be effective in treating a hard porous surface, some of the above recommended methods may be modified to simulate this more stringent condition by substitution of a porous surface carrier (such as a porcelain penicylinder or unglazed ceramic tile) for the non-porous surface carrier (stainless steel cylinder or glass slide) specified in the method. In addition, control data, described below in Supplemental recommendation No. (6), must be developed to assure the validity of the test results when this modification of the method is employed. Since the use of a porous surface would simulate the more stringent test condition, demonstrated efficacy on porous surfaces would suffice to support an analogous claim for efficacy on non-porous surfaces as well. In no case may a surface carrier which represents a less stringent condition be substituted for a surface carrier which is specified in the Recommended method.

(3) Hard water. The above recommended methods may be modified to demonstrate the effectiveness of an antimicrobial agent in hard water. The hard water tolerance level may differ with level of antimicrobial activity claimed. To establish disinfectant efficacy in hard water, all microorganisms (bacteria, fungi, viruses) claimed to be controlled must be tested by the appropriate Recommended method at the same hard water tolerance level. Refer to Recommended method No. 10 for the method of preparing hard water.

(4) Organic soil. An antimicrobial agent identified as a "one-step" cleaner-disinfectant, cleaner-sanitizer, or one intended to be effective in the presence of organic soil must be tested for efficacy by the appropriate method(s) which have been modified to include a representative organic soil such as 5% blood serum. A suggested procedure to simulate in-use conditions where the antimicrobial agent is intended to treat dry inanimate surfaces with an organic soil load involves contamination of the

appropriate carrier surface with each test microorganism culture containing 5% v/v blood serum (e.g., 19 ml test microorganism culture + 1 ml blood serum) prior to the specified carrier-drying step in the method. Control data, described below in (6), must also be developed to assure the validity of the test results when this modification is incorporated into the method. The organic soil level suggested is considered appropriate for simulating lightly or moderately soiled surface conditions. When the surface to be treated has heavy soil deposits, a cleaning step must be recommended prior to application of the antimicrobial agent. The effectiveness of antimicrobial agents must be demonstrated in the presence of a specific organic soil at an appropriate concentration level when specifically claimed and/or indicated by the pattern of use. A suggested procedure for incorporating organic soil load where the antimicrobial agent is not tested against a dry inanimate surface, such as the AOAC Fungicidal Test, involves adding 5% v/v blood serum directly to the test solution (e.g., 4.75 ml test solution + 0.25 ml blood serum) before adding 0.5 ml of the required level (5×10^6/ml) of conidia.

(5) Re-use. The recommended methods indicated in this section are designed to demonstrate efficacy of a freshly prepared antimicrobial solution intended for a single application. When the same use solution is intended for repeated applications, testing must be conducted in accordance with a test protocol specially designed to demonstrate retention of the claimed level(s) of antimicrobial activity in the use solution after repeated microbial and other appropriate challenges (such as organic soil or hard water) and stress conditions (such as inadvertant or incidental dilution inherent in the use pattern) over the period of time or number of times specified in the directions for use.

(6) Microorganism survival after drying on a hard surface. (i) Quantitative determinations of the microbial concentration on the untreated control carrier after drying are required in order to determine the validity of the test results obtained with the treated carriers when the recommended methods indicated above are modified to include such elements as:

(A) Test microorganisms not specified in the method;

(B) Substitution of a porous surface (e.g., porcelain penicylinder, unglazed ceramic tile) for the specified nonporous surface (stainless steel cylinder, glass slide); and/or

(C) An organic soil load.

(ii) The detailed protocol for this testing must include:

(A) Preparation of inoculum;

(B) Application of inoculum to the carrier;

(C) The time/temperature and relative humidity conditions for drying the microorganisms on the carrier;

(D) The technique for removal of the microorganisms from the carrier; and

(E) The specific assay procedure indicating such details as replication, subculture media/diluents, and the incubation time/temperature conditions for the enumeration procedure employed.

(iii) The test results must include the individual counts obtained by the method.

(7) Neutralization. For each antimicrobial product, procedures must be employed that will preclude residual effects of the active ingredient(s) in the subculture medium. A specific medium capable of neutralizing the antimicrobial effects of a product (whenever one is known) should be employed prior to the microbiological assay. Some of the recommended methods described in this section rely solely upon the selection of an appropriate subculture medium to neutralize the antimicrobial effects of certain general types of chemical compounds (active ingredients). (Refer to § 91-30 recommended method No. 7). However, to document the absence of residual effects of the active ingredient(s) in the subculture medium, the following testing is necessary:

(i) Secondary subcultures must be performed to demonstrate that antimicrobial effects were overcome; or

(ii) At the conclusion of the incubation period specified or employed in the method, the primary culture medium with test carrier must be inoculated with approximately 10 microorganisms/ml of the specific bacterial species under test (documented by actual plate counts) and reincubated for the specified period to demonstrate that the subculture medium was capable of supporting bacterial growth.

(8) Batch replication for modified tests. Where the required batch replication has already been performed and accepted for a product registration with unmodified tests by the recommended methods, additional testing at the same use concentration under modified conditions (e.g., different exposure period, presence of organic soil or hard water, porous surface carrier, etc.) may be conducted with reduced batch replication, as follows:

(i) For basic efficacy claims [e.g., sterilizers, § 91-2(a); disinfectants, § 91-2(b), (c), or (d); sanitizers, § 91-2(j) or (k)], two samples, representing two different batches instead of three.

(ii) For supplemental efficacy claims [e.g., fungicides, § 91-2(e); virucides, § 91-2(f); tuberculocides, § 91-2(g)], one sample instead of two.

Subseries 91B: NON-PUBLIC HEALTH USES

§ 91-51 General considerations.

(a) Scope. Sections 91-51 through -56 contain information concerning testing and performance of antimicrobial pesticide products for uses which are not directly related to human health. These uses include control of odor-producing bacteria, bacteria causing spoilage, deterioration, or fouling of materials such as paint or industrial fluids, and microorganisms infectious only for animals, where product failure against the specified pests would not have human health consequences. Pursuant to the efficacy data waiver provisions of Section 3(c)(5) of FIFRA, and § 162.18-2 of the FIFRA sec. 3 regulations, efficacy test data for these uses are not generally required to be submitted to support product registration [See § 90-1(b)]. Also, refer to § 101-1(b),(c),(d), and § 101-30 of Subdivision H for additional information concerning the relationship between label claims, human health considerations, and performance requirements for antimicrobial products. Requirements for testing and performance for those uses of antimicrobials which are identified as directly related to human health are provided in §§ 91-1 through 91-8 of this series. Labeling guidance for all uses of antimicrobial pesticides, both health-related and non-health related, are contained in §§ 101-1 through -16 of Subdivision H.

(b) General testing considerations. (1) In-use tests. Generally, demonstration of effectiveness of antimicrobial products in controlling microorganisms which are aesthetically or economically undesirable may be accomplished by establishing a correlation between successful control of the pest problem (e.g., odor, spoilage, fouling) and limitation of numbers of the target microorganisms at the site under actual conditions of use. In-use tests can be considered for any product of this kind on a case-by-case basis. However, field tests under an experimental use permit (refer to Subdivision I) are prescribed as a requirement only for the following non-public health uses:

(i) Antimicrobial fuel additives [see § 91-53(c)].

(ii) Antimicrobial additives for sugar mills [see § 91-53(d)].

(iii) Antimicrobial additives for poultry and livestock drinking water [see § 91-55(a)].

(2) Simulated-use tests. Except for the uses indicated in paragraph (b) of this section, simulated use laboratory tests can usually be considered as acceptable alternatives to actual in-use tests. Simulated-use tests should be designed to include the following basic elements:

(i) Identified test microorganisms (at least to the generic level) associated with the pest problem at specified site(s).

(ii) Appropriate surface(s) or substrate(s) which support growth of the test microorganisms under the environmental conditions (e.g., temperature, relative humidity) which simulate the in-use situation.

(iii) Adequately replicated test systems consisting of material inoculated with the test microorganisms and treated as directed with the antimicrobial product, together with parallel inoculated untreated controls.

(iv) Periodic observations on the presence or absence of the pest problem (e.g., odor, spoilage) which should include chemical, physical, or olfactory measurements.

(v) Parallel quantitative sampling techniques (e.g., agar plate counts) to enumerate the test microorganisms at appropriate intervals.

(vi) Conduct of the tests for a period of time which is recommended or required in actual use.

(3) Tests designed for public health uses. Effectiveness of antimicrobial products for certain uses in controlling microbial pests which are aesthetically undesirable (e.g., odor-causing bacteria) can often be extrapolated from the same kinds of efficacy tests required for public health uses (e.g., disinfectants, sanitizers, residual self-sanitizing treatments; see §§ 91-1 through -8 of this series) except for substitution of appropriate test microorganisms. Efficacy test data must be developed and submitted in accordance with human health uses (see §§ 91-1 through -3 of this series) when effectiveness is claimed or implied in labeling against microorganisms infectious for both man and animals. This is necessary to assure minimal protection of persons in contact with the animal environment. Qualified label claims against animal pathogens only would not generally require submission of specific test data against those microorganisms. When necessary [see § 162.18-2(d)(3)(ii) of the FIFRA sec. 3 regulations], the tests and performance criteria would be the same as those indicated for public health uses (§§ 91-1 through -8) except for substitution of appropriate test microorganisms.

(4) Qualitative screening tests. Qualitative data developed by presumptive screening tests, such as phenol coefficient tests, nutrient broth inhibition tests, or zones of inhibition on seeded agar or streak plates, are not considered to be of value in providing meaningful results that can be associated with end-uses of antimicrobial products and are unacceptable as documentation of efficacy for end-use claims. However, qualitative tests of this kind are acceptable to document potential or presumptive value of antimicrobial pesticide products intended only for formulation purposes (see § 91-57].

(5) Test substance. Unless othewise specified, products should be tested on the formulation as offered for sale and in accordance with the proposed directions for use.

(6) Neutralizers. In testing the efficacy of any antimicrobial product, appropriate neutralizers should be employed in the microbiological assay system, and evidence obtained to show that the neutralizers employed

inactivate the active ingredient(s) and do not possess any antimicrobial activity themselves. In lieu of specific evidence of chemical neutralization, it must be documented that appropriate secondary subculturing techniques have been employed that preclude residual effects of active ingredients in the assay medium. [Refer to § 91-30(e)(7).]

(7) Test variations. The protocol for testing will vary according to the type of product, type of substance to be treated, proposed use pattern, label claims, directions for use, and other factors peculiar to the specific product. In many cases, specific recommendations (such as the amount of replication) can be determined only after consideration of these factors. Refer to § 91-30(e) for guidance on some common test modifications (e.g., hard water, organic soil).

§ 91-52 Products for use on hard surfaces.

(a) Disinfectants (animal health). The following apply to all products represented in labeling as disinfectants for animal premises and equipment, including veterinary uses, farm uses, kennels, pet shops, zoos, and household pet areas.

(1) Control of microorganisms infectious for both man and animals: Public health uses. The efficacy data waiver provision § 90-1(b) is not applicable to microorganisms which are infectious for both man and animals. Unless disinfecting, germicidal, or bactericidal claims are specifically qualified as intended against animal and veterinary pathogens only, animal and veterinary premises disinfectants must be supported by basic efficacy data developed and submitted in accordance with the requirements for public health uses.

(i) Test standard. Same as § 91-2(b)(1), (c)(1), (d)(1), or (g)(1) of this series.

(ii) Suggested performance standard. Same as § 91-2(b)(2), (c)(2), (d)(2), or (g)(2) of this series.

(2) Control of microorganisms infectious only for animals: Non-public health uses. The efficacy data waiver provision § 90-1(b) is applicable to microorganisms which are infectious only for animals. However, the efficacy tests appropriate for such supplemental efficacy claims are the same as those which are required for public health uses, except for substitution of specifically claimed animal pathogens as test microorganisms.

(i) Test standard. Same as § 91-2(e)(1), (f)(1), (h)(1), or (i)(1) of this series, using specifically claimed animal pathogens as test microorganisms.

(ii) Suggested performance standard. Same as § 91-2(e)(2), (f)(2), (h)(2), or (i)(2) of this series.

(b) Odor control treatments (non-residual). The following apply to products represented in labeling as non-residual treatments to kill or reduce the number of odor-causing bacteria.

(1) Test standard. Same as § 91-2(b)(1), (c)(1), or (j)(1) of this series, except that pure culture isolates of identified odor-causing bacteria must be employed as test microorganisms.

(2) Suggested performance standard. Same as § 91-2(b)(2) or (c)(2) of this series for claims to kill odor-causing bacteria; same as § 91-2(j)(2) of this series for claims to reduce the number of odor-causing bacteria.

(c) Odor control treatments (residual). The following apply to products represented in labeling as residual treatments to reduce the number of odor-causing bacteria or bacteriostatic odor control in the presence of moisture.

(1) Test standard. Same as § 91-2(m)(1) of this series, except that pure culture isolates of identified odor-causing bacteria must be employed as test microorganisms.

(2) Performance guidance. Same as § 91-2(m)(2) for claims to reduce the number of odor-causing bacteria; for bacteriostatic odor control claims, the numbers of test microorganisms recovered from the treated surfaces should be less than the number recovered from the parallel control surfaces and no greater than the "0-time" control.

§ 91-53 Products for use on fabrics and textiles.

(a) Laundry additives. The following applies to antimicrobial products which bear label recommendations for treatment of laundry for odor control.

(1) Odor control pre-soaking treatments (non-residual). The requirements for products recommend to kill odor-causing bacteria on soiled fabrics by total immersion in the use solution prior to routine laundry operations are as follows:

(i) Test standard. Same as § 91-4(a)(1)(i) of this series, except that pure culture isolates of identified odor-causing bacteria must be employed as test microorganisms.

(ii) Suggested performance standard. Same as § 91-4(a)(1)(ii) of this series.

(2) Odor control laundry additives (non-residual). The following apply to products which bear label claims to kill or reduce the number of odor-causing bacteria when used in automatic or manual washing machine operations are as follows:

(i) Test standard. Same as § 91-4(a)(2)(i) or (a)(3)(i) of this series, except that pure culture isolates of identified odor-causing bacteria must be employed as test microorganisms.

(ii) Suggested performance standard. Same as § 91-4(a)(2)(ii) for claims to kill odor-causing bacteria; same as § 91-4(a)(3)(ii) for claims to reduce the number of odor-causing bacteria.

(3) Odor control laundry additives (residual). The following apply to products which bear label claims as laundry treatments to reduce the number of odor-causing bacteria or provide bacteriostatic odor control on treated fabrics in the presence of moisture when added to washing machine operations are as follows:

(i) Test standard. Same as § 91-4(a)(4)(i) of this series, except that pure culture isolates of identified odor-causing bacteria must be employed as test microorganisms. If claims are made for controlling development of ammonia odors from urine on laundered fabrics, Proteus mirabilis ATCC 9240 is required as the test microorganism and urea [1] must be added to test swatches.

(ii) Suggested performance standard. Same as § 91-4(a)(4)(ii) of this series for claims to reduce the number of odor-causing bacteria; for bacteriostatic odor control claims, the numbers of test microorganisms recovered from treated swatches should be less than the numbers recovered from the parallel control swatches and no greater than the "0-time" control; and for ammonia control claims, ammonia production should be delayed for the time period claimed.

(b) Carpet treatments. The following apply to products bearing label claims as carpet treatments to reduce the number of odor-causing bacteria.

(1) Test standard. Same as § 91-4(b)(1) of this series, except that pure culture isolates of identified odor-causing bacteria should be employed as test microorganisms.

(2) Suggested performance standard. Same as 91-4(b)(2) of this series.

(c) Mattresses and upholstered furniture. (1) Gases or vapors. The use of gases or vapor is currently the only effective and practical means of treating entire mattresses, upholstered furniture, pillows, and similar objects to kill or reduce the number of odor-causing bacteria. The following apply to products bearing such label recommendations:

(i) Test standard. Same as § 91-4(c)(1) of this series, except that pure culture isolates of identified odor causing bacteria should be employed as test organisms.

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[1] See: Latlief, M.A., M.T. Goldsmith, and J.L. Stuart. 1951. Germicidal and sanitizing action of quaternary ammonium compounds on textiles; prevention of ammonia formation from urea by Proteus mirabilis. J. Pediatr. 39: 730-737.

(ii) Suggested performance standard. Same as § 91-2(b)(2) or (c)(2) for claims to kill odor-causing bacteria; same as § 91-2(j)(2) for claims to reduce the number of odor-causing bacteria.

(2) Liquids. The use of liquid products applied by mechanical or pressurized spray for treating mattresses, upholstered furniture, pillows, and similar objects is an effective means of reducing the number of odor-causing bacteria on or in the ticking only. The following apply to products bearing such label recommendations:

(i) Test standard. Same as § 91-2(j)(1) of this series, employing ticking material instead of hard surface carriers as the test and control surfaces, and employing pure culture isolates of identified odor-causing bacteria as test microorganisms.

(ii) Suggested performance standard. Same as § 91-2(j)(2).

(d) Impregnated fabrics and textiles. The following apply to products intended for treatment of fabrics and textile materials, usually during the manufacturing process, to provide durable residual antimicrobial activity for reducing the number of odor-causing bacteria or bacteriostatic odor control on treated surfaces in the presence of moisture.

(1) Test standard. Same as § 91-2(m)(1) of this series, employing treated and untreated fabrics or fabricated items instead of hard surface carriers as the test and control surfaces, and employing pure culture isolates of identified odor-causing bacteria as test microorganisms.

(2) Suggested performance standard. Same as § 91-2(m)(2) of this series, for claims to reduce the number of odor-causing bacteria; for bacteriostatic odor control claims, the numbers of test microorganisms recovered from treated surfaces should be less then the numbers recovered from the parallel control surfaces and no greater than "0-time" control.

§ 91-54 Products for processing and industrial uses.

(a) In-can paint preservatives. Antimicrobial products which bear claims for use as preservatives in paint formulations are pesticides, and should meet the requirements indicated below. Paints containing preservatives are not pesticides unless pesticidal claims are made or implied.

(1) Test standard. Products proposed for use in preserving water-based paints should show effectiveness in controlling spoilage or deterioration caused by bacteria in at least two representative paint formulations in which the product is intended for use. Tests should be carried out in at least three replicates of each of the two paint formulations using pertinent microorganisms and adequate controls. Actual bacterial isolates (identified at least to genus) from spoiled paint and/or ATCC paint spoilage bacteria should be

employed as test inocula. Mixed bacterial and fungal inocula are not acceptable in demonstrating bacterial deterioration. Efficacy data should be derived from simulated-use type tests with quantitative bacteriological sampling and concurrent observations of paint quality. Both test and control samples should be tested for a period of six months to one year. The' test protocol, including such elements as frequency of repeated bacterial challenge, is contingent upon the intended preservative use pattern.

(2) Suggested performance standard. The data should show control of bacterial growth and control of bacterial-caused deteriorative (physical and chemical) changes in the treated paints during the test period. The data from control paints should show not only survival of test bacteria, but also significant growth and resultant deteriorative (physical and chemical) changes.

(b) Metalworking fluids. The following apply to products bearing label claims for preservation against bacterial growth and deterioration in metalworking fluids.

(1) Test standard. The product should be tested in one identified representative metalworking fluid formulation for each type (e.g., emulsifiable oil, semi-synthetic fluid, synthetic fluid) in which the product is recommended for use, and at the fluid-to-water ratio recommended in labeling. Three replicate tests should be carried out on each metalworking fluid formulation using appropriate controls. Each metalworking fluid formulation should be inoculated with a minimum of three different test bacteria. Each of the test bacteria should be identified at least to genus level. It should be documented that each of the test bacteria has been isolated from spoiled metalworking fluids of the type(s) in which the prduct will be tested or has been successfully employed to induce spoilage of such fluids in other tests. Either single, pure cultures of bacteria or a mixed bacterial inoculum may be employed. However, a mixed culture inoculum of bacteria and fungi is not acceptable. Although the control of microbial growth in metalworking fluids involves fungi as well as bacteria, fungal growth should be considered as a separate, though related control problem. Refer to §§ 93 (Efficacy of Fungicides and Nematicides) for information regarding the control of fungal growth. Each of the test bacteria should be present in the inoculum at a concentration at least 10^6 viable cells per ml of metalworking fluid. The tests should be carried out at a temperature of 25-28°c for periods of time with dosage amounts and intervals, and with fluid make-up procedures that are consistent with the recommendations for use on the label. Quantitative bacteriological sampling should be conducted with concurrent observations of fluid quality. Reinoculation with the test bacteria at regular intervals (e.g., weekly) to simulate repeated contamination/challenge to the system is necessary. The metalworking fluid in the control should be subjected to the same procedures.

(2) Suggested performance standard. The test should demonstrate control of deteriorative changes and inhibition of bacterial growth in metalworking fluids treated with the proposed product as recommended in labeling. The tests should also demonstrate, in metalworking fluids not treated with the proposed product, not only survival, but significant bacterial growth and resultant deteriorative changes. The results should include a report of

the physical or chemical changes observed in the fluids being tested.

(c) Antimicrobial fuel additives. The following apply to products bearing label claims for control of bacterial growth in kerosene based fuels (including jet aviation fuels) subject to water contamination, and diesel fuels or heating oils stored in metal tanks. With aviation fuel additives, the Federal Aviation Administration (FAA) should be consulted as to the acceptability of the additive from the standpoint of certification for particular airframes or engines.

(1) Test standard. (i) Laboratory test. The following basic elements should be incorporated into a presumptive laboratory test. A microbiological assay using Bushnell-Haas media plus the fuel (the fuel-to-liquid media ratio should be equivalent to that found in the field under actual conditions of use) inoculated with a mixed culture of bacteria and fungi (identified at least to genus) isolated from contaminated fuel and treated at the concentration recommended on the label. These data would presumptively determine the efficacy of a product.

(ii) Field test. (A) Aviation fuel additives. After presumptive efficacy is established as indicated in paragraph (c)(1)(i) of this section, products proposed for use in engines and/or airframes of aircraft should be field-tested according to the requirements specified in FAA Advisory Circular AC 20-24A, dated April 14, 1967, under an experimental use permit issued by the Agency. When an additive has not been certified for use in a particular aircraft engine and/or airframe, a disclaimer for such use must appear on the label.

(B) Other fuel additives. Any other proposed uses (diesel fuels, heating oils) would require field-derived efficacy data under an experimental use permit issued by the Agency after presumptive efficacy is established as indicated in paragraph (c)(1)(i) of this section.

(2) Suggested performance standard. The product should be shown to inhibit microbial growth in the presumptive laboratory test, and control the problems associated with microbial growth in the fuel systems employed in the field test. Federal Aviation Agency certification is required for aviation fuel additives.

(d) Antimicrobial additives for sugar mills. The following apply to products bearing claims for control of bacterial growth in sugar mill processes. Because cane-sugar and beet-sugar mills differ both in plant design and processing procedures, actual in-use testing should be conducted in both types of mills when products are recommended in labeling for use in both types.

(1) Test standard. (i) Laboratory test. Laboratory data showing the effectiveness of the product in inhibiting the growth of or reducing the number of representative *Leuconostoc mesenteroides* isolated from spoiled cane or beet sugar pressing should be provided.

(ii) Field test. Based on these data and on label recommendations, in-use testing should be conducted in at least one cane-sugar and/or one beet-sugar mill under an experimental use permit to demonstrate the efficacy of the product when used as directed. The basic elements which should be incorporated in the test protocols generally employed in the sugar mills should include the following: all chemical assays (e.g., Brix, invert sugar, lactic acid); all bacteriological assays based on plate counts, standard dilution methods, or other methods recognized as suitable by the industry (indicating time intervals and points of location in the systems where assay samples were taken); visual or other suitable rating of the control of bacterial slime accretion in the mill system; identification by genus and species if possible) of the isolated microorganism(s) which utilize sucrose; and the control treatment. The control treatment may be substituted with published information providing bacteriological data from untreated or inadequately treated systems, along with comparative bacteriological data from a comparable sugar mill treated with a formulation already registered for this use. Test reports should include, but are not limited to, the following: weight of raw cane or beets processed per unit time; product feed rate and/or concentrations used; the point or points in the mill system of product addition; location(s) and dates of the tests; and names (and titles or positions) of persons conducting the tests. Prospective registrants are reminded that a food-additive regulation or exemption from the requirement of such regulation under the Federal Food, Drug, and Cosmetic Act must be established before a product of this type can be registered.

(2) Suggested performance standard. The laboratory test should show that the product inhibits the growth of Leuconostoc mesenteriodes. The field test data should show the application of product according to label directions permits efficient operation of the mill system by reducing dextran deposits caused by the growth of sucrose-utilizing bacteria (i.e., L. mesenteroides) and that by maintaining the microbial population at an acceptable level, an increase in the yield of sucrose is realized due to the reduction of inversion losses.

(e) Miscellaneous preservative uses. In accordance with § 162.4(a) and (b) of FIFRA sec. 3 regulations, products that are recommended in labeling for use as non-food commodity preservatives are pesticides. Preservatives commonly bear claims to control bacterial spoilage or deterioration in such commodities as paper coatings, adhesives, plastic formulations, ceramic glazes, grouts, floor wax emulsions, gaskets (paper, felt, cork, rubber, vinyl), films and foams of polyvinyl and polyurethane, dextrin-based inks, photographic solutions, laundry starch, and colloidal graphite. Such products should be tested in each commodity claimed to substantiate effectiveness as a perservative. In accordance with § 162.4 (c) of FIFRA sec. 3 regulations, the preserved commodities themselves are exempt from registration.

(1) Test standard. Efficacy data should be derived from simulated-use tests with identified (at least to genus) spoilage bacteria. The tests should be carried out in triplicate using untreated controls with each commodity for a period ranging from several days to a year, depending upon the intended end use. Quantitative bacteriological sampling and concurrent observations of commodity quality should be performed.

(2) Suggested performance standard. For an effective treatment, the results should show inhibition of bacterial growth by quantitative techniques that can be related to colony-forming units with those microorganisms that have been isolated from the specific deteriorated substrate. Deterioration of the substrate in the untreated controls should be demonstrated, and the integrity of the treated substrate should be maintained and protected. The type of spoilage or deterioration which occurs in the untreated substrate should be described and documented.

§ 91-55 Products for control of microbial pests associated with human and animal wastes

(a) Self-contained toilet systems. Since it is ordinarily impractical to disinfect or sanitize human excrement in self-contained toilet systems by treatment with antimicrobial chemicals, the only pesticidal value attributable to such treatment is bacteriostatic odor control. The following apply to products bearing such label claims or recommendations.

(1) Test standard. Controlled in-use or simulated-use studies should be conducted comparing self-contained toilet systems treated with the bacteriostatic chemical with identical systems without the chemical. Quantitative bacteriological assay techniques, which can be related to colony-forming units, should be conducted periodically to evaluate inhibition of growth of the natural microflora contained in the waste of the treated system, when compared with growth in the untreated system. The test and control systems should be subjected to similar usage to provide meaningful data. The test protocol should incorporate a sampling schedule consistent with the time interval over which bacterial growth control is intended. Olfactory determinations comparing the development of odors in the test and control phases of the study should be performed simultaneously with the bacteriological determinations. The test should be conducted with an adequate control on each type of toilet system for which the product is intended for use.

(2) Suggested performance standard. The study should show that the product is effective in preventing the development of offensive odors during the time period that such control is intended. Bacteriological assays should demonstrate the inhibition of growth of microorganisms in the test system.

(b) Toilet bowl and urinal surfaces. The following apply to products bearing label claims to kill or reduce the number of odor-causing bacteria on toilet bowl and urinal surfaces.

(1) Test standard. Same as § 91-2(b)(1)(c)(1) or (j)(1) of this series, except that pure culture isolates of identified odor-causing bacteria should be employed as test microorganisms. Note that the contained bowl water (approximately 3 qts. or 96 fl. oz.) should be taken into consideration in determining the appropriate use dilution to be tested for toilet bowls.

(2) Suggested performance standard. Same as § 91-2(b)(2) or (c)(2) of this series for claims to kill odor-causing bacteria; same as § 91-2(j)(2) of this series for claims to reduce the number of odor-causing bacteria.

(c) Toilet and urinal bowl water. The following apply to products bearing label claims to reduce the number of bacteria or bacteriostatic control for odor, slime, or discoloration in toilet bowl water.

(1) Test standard. Same as § 91-7(b)(1) of this series, except that pure culture isolates of identified odor-, slime-, or discoloration-producing bacteria must be employed as test microorganisms.

(2) Suggested performance standard. Same as § 91-7(b)(2) of this series, for claims to reduce the number of bacteria; for bacteriostatic claims, the numbers of test bacteria recovered from the treated water should be less than the numbers from the parallel control and no greater than the "0-time" control; and for slime, odor or discoloration control claims, such problems should be delayed for the time period claimed.

(d) Bird and animal cage litter treatments. The following apply to products intended for application to or incorporation in pet bird and animal cage litter for bacteriostatic odor control in the presence of urine or wet fecal contamination.

(1) Test standard. Controlled in-use or simulated-use test should be performed to show the following:

(i) Numbers of bacterial contaminants in treated and untreated litter after initial deposition of actual bird and/or animal excrement and at periodic intervals thereafter (including repeated challenges with additional excrement) for the time interval recommended for use of the litter.

(ii) Olfactory assessment of the degree of odor control achieved over the same interval.

(2) Suggested performance standard. The numbers of bacterial contaminants in the treated litter should show a reduction over those in the untreated control, and the development of offensive odors should be reduced or delayed in the treated litter over the time interval claimed.

(e) Treated vomitus absorbents. The following apply to products intended for bacteriostatic odor control during clean-up and disposal of vomitus removed from inanimate surfaces.

(1) Test standard. Controlled in-use or simulated-use tests should be performed to show the following:

(i) Numbers of bacterial contaminants in treated and untreated absorbent after initial deposition of actual vomitus and at periodic intervals thereafter for the time period recommended or claimed for use of the absorbent to control odor.

(ii) Olfactory assessment of the degree of odor control achieved over the same period.

(2) Suggested performance standard. Same as paragraph (d)(2) of this section.

§ 91-56 Products for treating water systems.

(a) Drinking water for poultry and livestock. The purpose of the antimicrobial treatment of poultry and livestock drinking water should be clearly defined in labeling. Treatment of drinking water for the purpose of providing medication for animals, and/or implied claims of disease control, identify the product as a drug, and required approval by the Food and Drug Administration. The standards for products represented in labeling for treatment of poultry or livestock drinking water for pesticidal benefits (disinfection, sanitization, bacteriostasis) are considered below. Such products require a pesticide tolerance from the EPA under the Federal Food, Drug, and Cosmetic Act.

(1) Test standard. (i) Laboratory tests. Presumptive efficacy of poultry and livestock drinking water disinfectants or sanitizers may be established with data derived from the AOAC Method for Water Disinfectants for Swimming Pools (§ 91-30 Recommended method No. 14 in § 91 of this series) or with slight modifications thereof, against Escherichia coli (ATCC 11229) and Streptococcus faecalis (PRD). Presumptive efficacy for chemicals intended to provide bacteriostasis may be substantiated with any of several presumptive microbiological screening tests (e.g., minimal inhibitory concentrations derived from a broth tube-dilution type method, and zones of inhibition derived from a seeded agar cup plate type method).

(2) Field tests. Based on these data, controlled quantitative, microbiological studies should be designed to demonstrate the level of efficacy of the product in poultry or animal drinking water under actual conditions of use. Field-derived data should be developed under an Experimental Use Permit demonstrating the efficacy of the product when used as directed. Test conditions will vary with the level of effectiveness claimed, types of microorganisms to be controlled, application techniques for treating the water, treatment intervals, water dispensing system, type of animal facility, organic load, and other factors related to the proposed use.

(2) Suggested performance standard. The laboratory test should show elimination, reduction, or inhibition (i.e., disinfection, sanitization, bacteriostasis) of the test bacteria. Acceptable results for the field test will depend upon the level of activity claimed for specific use conditions.

(b) Potable water treatment units. Any unit intended for physical and/or chemical treatment of microbiologically potable water from a municipal treatment facility to remove undesirable taste odors, chemicals, or other aesthetically objectionable properties is identified as a potable water treatment unit. A substrate such as activated charcoal (with or without a bacteriostratic agent) is incorporated into the unit for this terminal processing treatment of potable water prior to consumption. Since the requirements of the Safe Drinking Water Act do permit municipally-treated drinking water to contain a limited number of harmless "saprophytic" bacteria which are commonly recognized contaminants of water, an antimicrobial agent is sometimes incorporated in a potable water treatment unit to provide bacteriostatic activity against these contaminants. Only potable water treatment units containing a bacteriostatic agent are under the purview of the Act.

(1) Test standard. Controlled, simulated-use studies for the potable water treatment unit should be conducted under conditions representing actual use, employing a defined municipally-treated water source. The test design of the study, which will vary for different types of units and patterns of use, should include the following basic elements:

(i) Evidence that the function of the potable water treatment unit (without a bacteriostatic agent) is impaired and/or adversely affected by identified microbial contaminants present in municipally-treated water, resulting in a recognized aesthetic problem (e.g., undesirable tastes or odors);

(ii) Quantitative determination of the level of microbial contamination in the test water before and after passage through the control (without a bacteriostatic agent) and test units;

(iii) Documentation of the bacteriostatic agent concentration found in the test system; and

(iv) Evidence of the effective capacity or duration of effectiveness of the bacteriostatic agent in controlling the contaminants responsible for the identified problem occurring under simulated in-use conditions.

(2) Suggested performance standard. The effective capacity or duration of effectiveness of the bacteriostatic agent incorporated in a potable water treatment unit should be established by meaningful results that can be associated with actual in-use conditions. The data should demonstrate that microbial contaminants in municipally-treated water cause a recognized aesthetic problem (e.g. undesirable tastes or odors) in the control units without a bacteriostatic agent, and that such problems are prevented or delayed in the test units with the bacteriostatic agent.

§ 91-57 Antimicrobial agents sold only for formulation use.

(a) Type of data. The manufacturer (or registrant) of a technical chemical intended for this type of use should submit presumptive evidence of intrinsic value as antimicrobial agent. Examples of the types of presumptive tests acceptable are the following: minimal inhibitory concentrations derived from a tube-dilution type method, and zones of inhibition derived from a seeded agar plate type method.

(b) (Reserved).

Series 92: EFFICACY OF AQUATIC PEST CONTROL AGENTS

§ 92-1 General considerations.

(a) Overview. A wide range of pesticides are used directly in aqueous environments or are intimately associated with them. These pesticides are used both in man-made systems, such as industrial cooling systems and swimming pools, and in natural aquatic areas, such as lakes and ponds. Applying pesticides to water can magnify unreasonable adverse impacts on man and the environment because contamination can extend to surface waters, ground waters, and aquifers used for, or in the production of, drinking, irrigation, or industrial process water. Subsequent contamination of plants and animals may lead to undesirable reduction of a species and economic losses. Bioconcentration of pesticide residues in the food chain or direct ingestion of pesticides by organisms in water may represent potential long-term hazards to man and the environment.

(b) Scope. Testing and performance guidance for the following products are stated in this section: aquatic herbicides, swimming pool algicides, industrial cooling water microbicides, pulp and paper mill water microbicides, secondary oil recovery systems microbicides, and antifouling paints. Mosquito larvae control agents are discussed under § 95-10 of this subdivision, and fish toxicants and fish repellents are discussed in § 96-2 of this subdivision. Applicants should also read the aquatic pest control agent label development discussions in § 103-1 of Subdivision H that relate to the specific pest, site, and proposed use-pattern.

(c) Considerations involved in efficacy testing. Guidance for establishing efficacy of aquatic pest control products discussed below in sections under specific product types (e.g., aquatic herbicides, antifouling coatings), are based on the following considerations:

(1) The intended or expected use of a pesticide-treated water.

(2) Dissolved and particulate constituents in the treated water, which may increase or decrease pesticide effectiveness.

(3) Those physical characteristics of the system which will affect volatility, absorption, dissolution, frequency, and efficiency of contact with the pest(s) to be controlled.

(4) Those physico-chemical characteristics of the water to be treated (such as temperature, pH, and hardness) which may affect efficacy.

(5) Application method(s), to enable the Agency to evaluate the practicability and potential selectivity of the proposed use, and to aid in the determination of proper classification of the pesticide according to § 162.11(c) of the FIFRA Sec. 3 regulations.

(6) Expected concentrations of pesticide in treated water as a result of treatment at the recommended dosage(s), to enable the Agency to evaluate the potential for nontarget effects and to aid in the determination of proper use classification.

(7) Phytotoxicity to crops or other nontarget vegetation expected to be exposed to the pesticide or to the pesticide-treated water, including total exposure of crops resulting from use in irrigation water, type of irrigation system, type of soil in which crop is grown, and distance of treatment site from crop.

(8) Indirect effects on nontarget organisms, including oxygen depletion and potential for resultant fish kills that may occur with use of aquatic herbicides.

(d) Relationship to use pattern. The test methodologies are organized according to use pattern. Different methodologies may be discussed even though a product is used to control one type of pest, such as algae in several sites. Differences between pests, sites, or application techniques can affect the efficacy of a product. Accordingly, in most cases, data demonstrating the efficacy of a product will be acceptable only if such data are derived from the methodology appropriate to that product's specific use pattern.

§ 92-2 Aquatic herbicides.

(a) Scope. This section provides guidance for pesticides designed to control aquatic macrophytes (mosses and vascular plants), ditchbank plants, and algae. Efficacy data concerning control by pesticides in swimming pools, industrial cooling water systems, pulp and papermill water systems, secondary oil recovery systems, and on ship or boat bottoms, as well as other underwater surfaces, are discussed in §§ 92-3, -4, -5, -6, and -7.

(b) General data conditions. The following conditions should be met in order to establish efficacy of aquatic herbicides and algicides covered in this section (§ 92-2) and effects of these pesticides on the environment, whether they are intended for use in standing or moving waters, adjacent to water, or in a location expected to contain water at a later time. Additional conditions which pertain to specific categories are covered as follows: standing water, moving water, ditch bottoms, and ditchbanks [paragraphs (c), (d), (e), and (f), respectively, of this section]. Field testing should include a geographical distribution of plot sites that reflects the general climatic and water quality variations expected in the locations where the product is proposed for use. Not all such environmental variations can be anticipated or tested, but sites shall include those which have various water hardness, pH, temperature, turbidity, alkalinity, and weed infestations (both in types and numbers of weeds). Field tests should be designed to allow statistical comparison of weed control between treated and untreated ("control" or "check") plots. Whenever possible, a known "standard" treatment should be included in the testing. For example, when testing a new algicide, copper sulfate may be used for a standard. For these comparisons, the number and kind (species) of target weeds present in the control and treated plots should be similar in extent and type of weed, in relative species abundance, and in water quality. The design of such plots is discussed in the Acceptable Method No. 3 § 92-20(d). (Efficacy Testing Methods for Aquatic Herbicides in Standing Waters). In all cases, the following data should be reported for each test site.

(1) Description of test site. This includes the following: (i) Type of aquatic site, such as lake, pond, reservoir, ornamental pool, irrigation ditch, and geographic location of site (i.e., state, county, town).

(ii) Size (area and depth, or volume, or length and width of the treated areas, and of the whole site), as is appropriate to the type of application and the type of target weed(s).

(iii) Number of replicate treated plots and control plots.

(iv) Water quality, including pH, temperature, hardness, alkalinity, salinity, turbidity, conductivity, and dissolved oxygen.

(v) Soil texture, including that of soils along the immediate shoreline or ditchbank and the submersed soil where the target weeds are present (with the percent organic material in the soil also reported). (Acceptable methods and soil texture classifications are found in the Walkley-Black Procedure in Soil Sci. 63:251, 1947, and the Soil Survey Manual, U.S. Dept. Agr. Handbook No. 18, 1951, Fig. 1, and Soil Sci. Soc. Amer. 26:305-317, 1962.)

(2) Description of vegetation present at test site. Description should include the vegetation present and any organisms that affect product performance. The following shall be reported:

(i) Target weed species (common name if available, and scientific name), and weed density (number or amount per unit area or per unit volume, as appropriate).

(ii) Other vegetation (common name or scientific name).

(iii) Growth stage of target weeds, such as seedling, preflowering, or heading stage.

(iv) Other appropriate observations relating to target weeds, such as the presence of a heavy coating of epiphytic algae on the leaves of the target vascular plants.

(3) Methods of herbicide application. Information should include:

(i) Target site where the herbicide was applied (for example, to weed foliage, to surface of water, to bottom of water body, into water, to ditchbank, or to shoreline).

(ii) Description of the equipment used to apply the herbicide (e.g., ground-spraying device, pumping device, boat, blower, helicopter, or fixed-wing aircraft).

(iii) Description of any water level changes used in conjunction with the herbicide application, such as drawdown operation or drainage of conveyance system, including the extent of water level change, the time of the change in relation to the herbicide application, and the duration of the change in water level.

(iv) The timing of the application in relation to the calendar date and the stage of growth of the target weeds.

(4) Dosage of herbicide. The dosage ranges tested should be broad enough to determine the minimum efficacious dosage and the effective range for each target weed claimed. In addition, the toxicity to desirable nontarget vegetation should be determined if it is within the dosage ranges tested. (Refer to Subdivision J of these guidelines for guidance and acceptable methods in determining phytotoxicity to nontarget plants.) The following conditions should also be known:

(i) The amount of product and active ingredients should be expressed in units appropriate to the method and placement of the application, such as weight per unit area, weight per unit volume, or the weight per unit flow-volume, and, in every case, the concentration of active ingredient in the water.

(ii) Sufficient variety of soil textures and water quality conditions should be used to determine the effective minimum dosage or dosage range for the particular soil and water conditions likely to be encountered in the geographical area where the use is proposed. The broader the geographical area of proposed use, the more tests will be needed. Generally, in any one locality or geographical area (for example, the southeastern United States), a minimum of three testing sites will be needed, but six or more are strongly recommended. The acceptability of the number of tests will ultimately depend on the quality of the data.

(iii) If adjuvants such as surfactants, "spreaders", or "stickers" are required for proper efficacy, appropriate adjuvant control plots (without herbicide) should be included to distinguish the possible physiological or phytotoxic effects of the adjuvant alone.

(5) Limitations on water use for irrigation. If label claims intend that the product will be used to treat water that will be a source for irrigation water, or to treat ditchbanks in irrigation systems (including drainage ditches), then the following are necessary:

(i) Test(s) should be conducted to determine if phytotoxic effects of the herbicides occur on crops normally expected to be irrigated with treated water, and to determine the highest concentration of the product in water which will not injure crops or desirable vegetation expected to be exposed. (See § 92-20(c) for references on methods for determining phytotoxicity of aquatic herbicides to irrigated crops).

(ii) To evaluate potential phytotoxicity to crops as a result of cumulative effects of herbicide residue in irrigation water, the following information for anticipated crop practices are needed: frequency of irrigation, amount of water per irrigation treatment, total acreage treated per crop season, and concentration of herbicide in irrigation water when treated water reaches crop.

(iii) Concentrations of the active ingredient in water as result of proposed application should be predicted, as well as the decline of that concentration with time and/or distance of flow. Appropriate statistical analyses of the data and development of depletion curves are essential. (See § 92-20(a) for references on dissipation analysis methods; also, see §§ 164-1 through -5 of Subdivision N.)

(6) Results of aquatic herbicide treatment(s). In this subparagraph, guidance for reporting results of tests on any aquatic herbicide are stated first. Additional guidance for aquatic macrophytes and algicides are discussed in (ii) and (iii) of this subparagraph (6).

(i) Tests results for all aquatic herbicides should include:

(A) The observation dates, with the time interval(s) between initial applications and subsequent observations;

(B) The plant species controlled, and the duration of control for each species in days, weeks, or other time period;

(C) Comparison with "standard" herbicide treatment plots (if used);

(D) Herbicide phytotoxic effects on target weeds and other vegetation in the test plot. The indicative visual phytotoxic symptoms of the target weeds and the time of their appearance are particularly important if subsequent herbicide treatment(s) or the manipulation of water levels or water flow is dependent upon the knowledge of the effectiveness of initial applications of the herbicide. Examples of terminology for such symptoms appear in § 102-2 of Subdivision H;

(E) Results of crop phytotoxicity tests (if crops are expected to be exposed to treated water), and the maximum level of the herbicide tolerated by crops expected to receive treated water, including any limitations of the type of irrigation and on timing of irrigation after water treatment; and

(F) Changes in water quality following herbicide treatment and weed control. [This includes those analyses specified under paragraph (b)(1)(iv) of this section.]

(ii) Test results for aquatic macrophytes (including ditchbank plants) should also include the extent of weed infestation before and after herbicide treatment(s) in both control and treated plots, and the calculated percent weed control for each weed species. Data should be based on appropriate measurement(s) of weed species population density and may include dry or wet weight of weeds, length of weeds, and height of weeds, depending upon the type of control and the type of weed.

(iii) Test results for algae should also include:

(A) Amount of growth present (for example, density per unit area or per unit volume of water) before and after treatment(s). Methods for evaluating algae control are discussed in § 92-20(d) Efficacy Testing Methods for Aquatic Herbicides in Standing Waters; and

(B) Changes in the degree of infestation of other aquatic plants following target algae control.

(c) Specific data guidance for standing water. In addition to the guidance given in paragraph (b) of this section, the following are needed:

(1) Dosage of herbicide. (i) For applications to aerial foliage, the amount of herbicide used per unit area, or per total spray volume, or both.

(ii) For applications where effectiveness is dependent upon concentration in the water, the dosage used in amount per volume [e.g., hectare-meter (acre-ft.)], and the concentration in water (e.g., parts per million) resulting from the application.

(2) Hydrological features. Those hydrological features which can alter herbicide usefulness, such as thermoclines (depth and steepness), inflows which may reduce initial concentration levels, and wave action.

(d) Specific data guidance for moving and flowing waters. Herbicides that are applied directly to moving water (for example, canals, ditches, irrigation systems, streams, rivers) are included here. In addition to the guidance given in paragraph (b) of this section, the following are needed:

(1) Dosage of herbicide. (i) For herbicides whose effectiveness is related to concentration in water, the amount of herbicide per unit volume of water per unit time and its dissipation with time. [See § 92-20(b) for information on method.]

(ii) The most effective concentration and contact-time combination(s) for the target weed.

(2) Flow characteristics. The flow rate (flow volume) in the conveyance system (e.g., cubic feet per second).

(3) Construction material. The type of construction of the treated conveyance system (for example, earthen or concrete-lined).

(4) Previous and subsequent comparisons. Duration of weed control and comparison with weed infestation during previous years, when known.

(5) Untreated control. Untreated plots should be located upstream from treated plots at a sufficient distance to preclude pesticidal effects. If there is no appropriately similar site upstream, comparison before treatment and after treatment should be made.

(e) Data guidance for ditch bottoms. For treatments to canal or ditch bottoms, the following should be reported, in addition to paragraph (b) of this section:

(1) Weather conditions. Type and amount of precipitation, and general weather conditions between application time and time when water is turned into the canal.

(2) Draining interval. Time interval between drainage and application, and between application and time when ditch or canal is refilled.

(3) Amount of control. The length (meters, kilometers) of canal treated, and length of achieved weed control in the canal.

(4) Herbicide residue. The concentration of the herbicide in water after it is turned into the drained canal, if the water is to be used for irrigation purposes. (See § 164-2 of Subdivision N for analysis techniques.) Other guidance related to use of treated irrigation water are described under paragraph (b)(6) of this section.

(f) Specific data guidance for ditchbanks. For ditchbank treatments, the extent of water overlap, if any, should be stated so that the amount of exposure of water can be calculated and correlated with the analyses of concentrations in the water.

(g) Performance standards. Normally, the degree of aquatic weed control that is acceptable depends upon the particular use site and use pattern. The calculation of percent weed control should be based on weed biomass, height (or length) of weeds, number of weeds, or amount of surface area covered by weeds. Other assessments may be utilized if they reflect beneficial effects of weed control. When percent weed control is based on comparisons of untreated plots to treated plots, or to pretreatment weed infestations in treated plots, a minimum of 70% control is desirable. Lesser effectiveness may be acceptable, provided that appropriate qualifying terminology is used on the label. [See § 102-2 of Subdivision H. Specific standards for performance are stated below for the indicated uses.

(1) Algae control. For laboratory testing using pure-culture methods to obtain presumptive efficacy data, a minimum of 70% control is needed. To support algicidal claims, subcultures should not show growth within 21 days after inoculation into fresh medium. For field tests, the following degree of control should be obtained:

(i) Prevention of growth (bloom) by at least 80% compared to untreated plots, or

(ii) Reduction of existing growth (bloom) by at least 70%, or

(iii) Indirect performance results, such as improvement of volume-flow in canals or ditches by at least 50%, reduced clogging of pump screens by at least 70%, and reduced clogging of drip-irrigation systems by 95%.

(2) Aquatic macrophyte control. (i) Standing water. A minimum of 70% control as measured by weed count, biomass, length, area, or other appropriate value. For floating weeds, the percent cleared water may be used as an evaluation, and ordinarily should be at least 70% of the treated area.

(ii) Moving water. At least 70% control of submersed or emersed weeds, or an increase in flow-volume by at least 50%.

(3) Ditchbank weeds. At least 70% control and lesser control will be acceptable only if appropriate qualifications are stated on the label.

§ 92-3 Swimming pool algicides.

(a) Scope. Two types of tests are used to demonstrate effectiveness of swimming pool algicides to control algae: laboratory tests using pure-culture methods [see paragraph (b) of this section], and confirming tests [see paragraphs (c) and (d) of this section] in swimming pools or simulated swimming pools. If an experimental permit is needed, data from laboratory pure-culture testing is needed. For tests to determine efficacy of pesticides to disinfect or control disease organisms in swimming pool water, see § 91-8(c) of this subdivision.

(b) Laboratory tests. Laboratory procedures are necessary to assess the algistatic versus algicidal capabilities of these products. An acceptable laboratory pure-culture method is given in § 92-20(e) Pure-culture Technique for Evaluating Algicides.

(c) Confirming in-use tests. Data developed from tests conducted by either of the following methods are acceptable.

(1) In-use pools. Along with adequate laboratory data, confirming efficacy data are recommended from in-use tests in two or more properly maintained swimming pools of different sizes located in different geographical regions of the country. The tests should be conducted for a period of not less than 30 consecutive days under climatic conditions occurring during the swimming pool season. Reports should include but are not limited to the following additional information on the condition of the test pool:

(i) The design and capacity of the pool.

(ii) The pool use and bather load.

(iii) The amount of product added to swimming pool water and the time of addition.

(iv) The range of chemical characteristics of the swimming pool water, such as pH, nitrogenous substances, alkalinity, hardness, and amount of residual disinfectants and stabilizer(s).

(v) The physical characteristics of the swimming pool water, including temperature, color, and clarity.

(vi) Meterological data, including air temperature, rainfall, and number of hours of sunlight, which may affect results.

(2) Simulated pools. In lieu of the in-use tests, acceptable confirming data may also be obtained from tests conducted in simulated swimming pools artificially inoculated with algae. Consultation with the Agency about procedures and test species prior to undertaking these tests is strongly recommended. An acceptable simulated swimming pool method is provided in § 92-20(f) Method for Evaluating Algicides in Simulated Swimming Pools.

(d) Suggested performance standards. (1) Pure-culture tests. For pure-culture testing, the acceptable level of performance is 70% control of algae in primary cultures for 3 weeks. (See § 92-20(e)).

(2) In-use pools. For in-use swimming pool testing, the level of performance should equal or exceed that of a standard chlorine treatment.

(3) Simulated pools. For simulated-swimming pool testing, the acceptable level of performance must be equal to or better than a chlorine standard at 1.0 to 1.5 ppm for isocyanuric acid-stabilized pools or at 0.6 to 1.0 ppm for non-stablized pools. (See § 92-20(f)).

§ 92-4 Industrial cooling water microbicides.

(a) Scope. Products intended to control microbiological slimes in cooling water systems include those with algicidal, bactericidal, and fungicidal activity. Microbiological slimes consist of microorganisms (algae, bacteria, and/or fungi) and their secretions, plus embedded debris.

(b) Definitions. The following definitions and explanations are particularly pertinent to the understanding of this section:

(1) Bactericide. As used in this section, this term is restricted to products used in cooling water for controlling bacterial slime, and must not be construed to include products represented or defined as disinfectants described in §§ 91 (Efficacy of Antimicrobial Agents).

(2) Blowdown (bleed-off). This term means: water discharged from the system to control concentrations of salts or other impurities in the circulating water. Blowdown is measured in volume per unit time.

(3) Closed recirculating systems. This term means: a system of continuously recirculating water in a heat exchanger that may be cooled by air, mechanical refrigeration, or a separate open cooling-water system.

(4) Makeup water. This term means: water added to the circulating water system to replace that lost because of blowdown (bleed-off), drift, evaporation, leakage, and overflow.

(5) Microbicide. As used in this section, this term means: any substance or mixture of substances which effectively reduces the number of algae, bacteria, and/or fungi.

(6) Once-through systems. This term refers to systems where water is taken from some primary source, used for cooling, and then either discharged to waste or used for other purposes.

(7) Open recirculating systems. This term refers to systems where a major portion of the water used for process cooling is continuously recycled to some type of evaporative cooling device, such as a cooling tower, to remove the heat picked up in the process, and is then used again for process cooling.

(c) Data guidance. (1) Laboratory testing. Laboratory data should be submitted to show presumptive effectiveness of the product in controlling algae, bacteria, fungi or combinations thereof when field testing is used to evaluate product performance.

(i) Standard laboratory techniques should be used to determine minimum concentrations of the product which will kill or prevent growth of target organisms. The viability of pure culture algae should also be determined by a technique involving subculture into untreated medium. (See § 92-20(e)). Laboratory evaluation of bacteria and fungi shall consist of determining minimum effective static and cidal concentrations of product on unshaken liquid cultures.

(ii) The following test species should be employed: bacteria: Pseudomonas spp. and Bacillus mycoides (ATCC); fungi: Aspergillus niger (ATCC); algae: Chlorella pyrenoidosa, Euglena gracilis, and Scenedesmus obliquus. (For algae, see § 92-20(e) for methods and growth media).

(d) Field testing. Tests should be made in at least 2 different climatic locations for a period of not less than 30 days. Test systems should be representative of the more severe conditions that the product is intended to control.

(1) Reports should include, but are not limited to, the following:

(i) Dosage applied at each treatment and contact period necessary for control of organisms.

(ii) Interval between treatments.

(iii) Visual or other suitable ratings of the nature and amount of microbial growth. The rating scale shall be defined, and the commercially acceptable level of control should be indicated.

(iv) Total volume in the system.

(v) Percent blowdown, if applicable, per unit of time.

(vi) Water characteristics, such as pH, hardness, temperature; identification and estimate of concentration of any anticorrosion inhibitors in the system; identification of any antimicrobial chemicals that were used immediately before or during the test period.

(vii) Engineering data, if available and if appropriate for comparative purposes, reflecting the relative efficiency of operation of the system, such as heat transfer readings, head pressures, and down time. Baseline data on each are necessary for comparative purposes.

(viii) Microbial counts before and after each treatment to support the claimed interval(s) between applications and to indicate the degree of control obtained.

(ix) For solid products such as briquettes and tablets, data must be submitted showing the rates of dissolution of the product in water at various temperatures and flow rates.

(x) Where and when the tests were conducted.

(xi) Background and training of person(s) conducting test.

(2) Foreign data. Similar field data developed in foreign countries are acceptable if the data have been obtained from two cooling water systems where climatological conditions and the microorganisms encountered are similar to those found in cooling water systems in the United States.

(3) Laboratory evaluation of cooling tower bacteria and fungi. In lieu of field testing, water samples from operating cooling towers containing indigenous microorganisms can be tested by subculture and plated onto surface medium for enumeration. Algicidal or algistatic activity will be determined by specified laboratory testing. See ASTM Method E-645: Test for Efficacy of Microbicides Used in Cooling Systems. 1/

(4) Laboratory model systems. Laboratory methods for efficacy evaluation of cooling system microbicides will be considered on an individual basis. Registrants are advised to submit such proposed laboratory methods to the Agency for review and approval prior to their use in developing support data for registration.

(d) Suggested performance standards. (1) Laboratory testing. For laboratory methods used in lieu of field tests, a minimum of 90% bacterial kill within a 3-hour contact period with the product is desirable. Growth of fungi should be prevented. Performance standard for algae is 70% control of algae growth in primary cultures for 3 weeks. (See § 92-20(e) Pure-culture Technique for Evaluating Algicides.)

(2) Field testing. Application of the product according to label directions during field tests should permit efficient operation of cooling systems by controlling biological fouling so that proper compressor head pressures and temperature differentials are maintained.

§ 92-5 Pulp and papermill water systems microbicides.

(a) Residues in paper. Treatment of pulp and papermill process water with pesticides may result in residues in finished paper or paperboard. Residues occurring in finished paper products intended for use in contact with food must be covered by food additive regulation or by an exemption from the requirement for such regulation. (Refer to § 63-1.)

(b) Data guidance. Laboratory data should be developed to show the effectiveness of the product against both bacteria and fungi by the ASTM "Tentative Methods of Test for Efficacy of Slimicides for the Paper Industry." 2/ Two individually formulated samples of the microbicide developed be tested separately, in duplicate, against bacteria and against fungi, for a total of four tests. For solid formulations, data should be developed showing the rates of dissolution of the product in water at various temperatures and flow rates. Field data from 2 operating mills in the United States will be acceptable. Similar data developed in foreign countries are acceptable if the data have been obtained in 2 mills where climatological conditions and the microorganisms encountered are similar to those found in mills in the continental United States.

(c) Suggested performance standards. (1) Bacterial control. The Agency will accept the performance standard described in the ASTM "Tentative Methods of Test for Efficacy of Slimicides for the Paper Industry" for bacterial control.

(2) Fungal control. The performance rating system for control of fungi assessed by the ASTM "Tentative Methods for Test for Efficacy of Slimicides for the Paper Industry" is as follows:

0 - no growth

1 - scant, or questionable growth

2 - poor growth

3 - good growth

4 - excellent growth

The level of performance can be indicated with the use of a standard reference microbicide.

§ 92-6 Secondary oil recovery systems microbicides.

(a) Methodology. The American Petroleum Institute (API) "Recommended Practice for Biological Analyses of Subsurface Injection Water Tests" 3/ contains an acceptable method for assessing the efficacy of products against *Desulfovibrio desulfuricans*, *Bacillus cercus*, and *Pseudomonas fluorescens*. The bacteriostatic and time-kill test must be carried out in duplicate.

(b) Suggested performance standard. The product tested as specified by the API method must prevent growth of test bacteria.

§ 92-7 Antifouling biocides.

(a) Scope. Antifouling coatings fall into two main usage categories: marine and freshwater. Many marine paints will not perform satisfactorily in fresh water. To some degree, the reverse is also true. The main testing emphasis has been in the marine environment. To some extent, marine and freshwater paints are used to prevent fouling on substrates other than boats or ships: surfaces on submersed equipment such as irrigation wiers, power plant intake or outflows, and pipe lines, are often protected in both environments by antifouling coatings. But the major use of these coatings is on ship and boat bottoms. Control of all fouling, be it algal or invertebrate, is desired. All non-biocidal aspects of coatings, such as color and friction reduction, are outside the scope of pesticide regulation.

(b) Testing considerations. (1) General. Sward (1972) 4/ points out that tests should start early in the spring, and that panels should be exposed at widely-separated geographical points. The USNI (1952) 5/ advises that panel tests are the most valuable and oldest of the testing methods used, but must be conducted in locations when the larvae of fouling organisms are present throughout the year. Panels coated with an antifouling paint of proven performance should be included in all tests to serve as "standards". Since some variation in standard performance will occur depending upon conditions at each test location, such standard antifouling paint panels are essential for comparison purposes. The use of standard panels coated with U.S. Navy Formula 121/63 (MIL-P-15931B) antifouling paint is strongly advised. The use of a nontoxic untreated "control" panel is required to properly evaluate the fouling potential. The standard panel gives a known performance rating and can be used as a high performance standard. The untreated panels give a survey of the fouling community that would be noted if no toxicant were present in the paint under test, or if the toxicant were ineffective.

(2) Data guidance. Antifouling paints or coatings should be tested for periods of one or more "seasons" in the locations of intended usage, depending upon intended label claims. The length of a "season" may vary from six months to one year, depending on the location of the test site. The information and data that should be recorded and reported are enumerated below:

(i) Substance of test panels, i.e., wood, steel, aluminum, or other material.

(ii) Painting system used for undercoat (including panel preparation).

(iii) Method of application of paint being tested (brush, spray, or roller).

(iv) Wet or dry film thickness, weight of film applied, and/or number of coats.

(v) Drying or curing time before immersion at test site.

(vi) Formulation used, including percent active ingredient.

(vii) Panel size and number of replications. A minimum of four test panels per condition or paint to be tested should be employed, i.e., four standard panels, and four untreated control panels. The surface area of each panel should contain a minimum of 46208 sq mm (72 sq in.), or be at least 152 by 304 mm (6 x 12 in.).

(viii) Location of test site.

(ix) Conditions encountered during test period (such as average water temperature, salinity, pH, hardness, turbidity, and sediment).

(x) Application date(s).

(xi) Immersion date and method of exposure when:

(A) Suspended from a float, a fixed support, recording depth at lowest tide.

(B) Partially immersed, for evaluating waterline fouling.

(xii) Termination data and period of acceptable control (effective life of coating).

(xiii) Monthly readings of fouling during the "season" of paint exposure. Identity of algae, barnacles, tunicates, or other fouling organisms as to genus and species is not necessary; however, if such identification is possible, it should be reported. Readings should include data enumerating the discrete numbers of individual organisms or the percent area occupied by colonial forms for each panel observed and for each type of organism. Control and standard panels should be rated in a manner identical to that for test panels.

(3) Evaluation of data. Fouling rating: for each observation a Fouling Resistance (F.R.) and an Antifouling Film Rating (A.F.) should be developed. Acceptable rating systems are presented as follows:

(i) Surface free of attached fouling organisms = score of 100.

Substract from 100 the total number of individual organisms present, or the percent area covered by colonial forms, for each panel observed. For example:

Barnacles - 5 each	5
Tunicates - 2 each	2
Algae - 7 percent	7
(Total)	14

	100
	-14
Fouling Resistance (F.R.) =	86

(ii) For test films, the Antifouling Film Rating (A.F.) is developed by subtracting the percentage of the surface showing apparent defects (pits, cracks, checks, peeling, flaking, etc.) from 100. A film with no defects scores 100.

(4) Suggested performance standard. For an antifouling coating to be considered acceptable, the test panels at the end of the test should still show at least 85% freedom from attached fouling organisms. Panels rated under 85% "F.R." for the time period claimed by the label are considered failures.

§ 92-10 Footnotes to Series 92 sections.

1/ Copies of E-645 may be obtained from the American Society for Testing and Materials, 1916 Race St., Phila., Pa. 19103.

2/ Copies of the current test procedures may be obtained from the American Society for Testing and Materials, 1916 Race Street, Philadelphia, Pa. 19103: E-599 for fungal method, and E-600 for bacterial method.

3/ API RP 38, 3rd Ed., Dec., 1975. Copies of this test procedure may be obtained from the American Petroleum Institute, Division of Production, 300 Corrigan Tower Building, Dallas, Tex. 75201.

4/ Sward, G.G., ed. 1972. Paint for Marine Environment. Pp. 478-485 in Paint Testing Manual, 13th Ed. Amer. Soc. for Testing Materials, 1916 Race St., Phila., Pa. 19103.

5/ Anon., 1952. Marine Fouling and its Prevention. U.S. Naval Institute, Annapolis, Md. 21402.

§ 92-20 Acceptable methods.

(a) Introduction. The discussions that follow describe acceptable methodologies for obtaining efficacy data in support of registration applications for aquatic pest control products. Where methods are specifically delineated, the Agency has judged that these procedures will provide the necessary data for evaluation of efficacy. In other cases, reference is made to published literature as a guide for developing acceptable test methodologies. More than one type of referenced study may have to be utilized to meet the efficacy data requirements as stated in the guidelines, on Aquatic Pest Control Agents. The uses of aquatic pesticides are so varied that it is impractical to provide detailed testing protocols for many product types; however, if the cited references are consulted and the data referenced in these guidelines and those on labeling requirements (Subdivision H, §§ 102-1 to -6 are carefully read, the applicant will be able to carry out acceptable testing. If there are questions regarding a particular test procedure that the applicant intends to use, the applicant or tester should confer with the Agency prior to testing. Testing to meet both general and specific data guidance is discussed in the following paragraphs.

(b) Dissipation analysis methods. (1) General. The intent of these studies is to obtain in-use field data on the concentration of herbicides in water that will contact crops when the herbicide is applied in any of the following sites:

(i) Directly to or in water used as an irrigation source.

(ii) Drained irrigation conveyance systems.

(iii) Drawdown impoundments used for holding water intended for irrigation.

(iv) Ditchbank applications to irritation water conveyance systems or shoreline application to irrigation water holding impoundments.

(v) Multi-use aquatic sites for which irrigation is an expected use.

(2) Use of data. The dissipation behavior of herbicides applied to sites (i) through (v), mentioned in paragraph (a) above, should be known to accurately assess the potential phytotoxicity of the herbicides to crops irrigated with treated or exposed water. The information is used in conjunction with testing of crop phytotoxicity. [See paragraph (c), "Methods for Testing Crop Phytotoxicity of Aquatic Herbicides"]. The data derived from these tests are used to develop appropriate precautionary labeling to insure that aquatic herbicides will be used without injury to crops. For this reason, the dissipation testing must allow the prediction of crop exposure to herbicides as a result of the treatment.

(i) Data guidance for application of herbicides to standing waters, or shorelines thereof:

(A) The time (hours, days, weeks) necessary for herbicide concentrations to decline to pre-application levels at the site of application and at the closet point of intake into the irrigation system;

(B) The minimum distance from application site to point of intake into the irrigation system;

(C) For drawdown application, the minimum time required for concentration of herbicides in re-elevated water to decline to preapplication levels and the minimum distance from application site, if any, to point of intake into irrigation system.

(ii) For application to irrigation ditchbanks or water in irrigation conveyance systems:

(A) The time (hours, days, weeks) required for herbicide concentration in the water to decline to pre-application levels at the point where maximum levels are reached and at the point where treated water first contacts crops;

(B) The minimum safe distance from application site to point where treated water first contacts crops.

(iii) For application to drained irrigation conveyance systems:

(A) The minimum time required for herbicide levels in reintroduced water to decline to pre-application levels in water prior to draining at the site of application and at the point of first contact with crops;

(B) The minimum safe distance, if any, from application site to point of first contact of reintroduced water to crops.

(iv) Acceptable methodologies. Simple and direct methods for determining the dissipation of several phytotoxic chemicals in flowing water are published (Bartley, 1967, 1969; Chancellor et al., 1957; Demint et al., 1970; Demint, 1971; Frank et al., 1967; Nelson et al., 1969). Some further theoretical considerations of herbicide applications to moving water, and some examples of dissipation data are found in O'Loughlin and Bowmer (1975). Examples of field analysis for dissipation in standing waters are found in Grzenda et al., (1966), Hiltibran et al., (1972); Simisiman and Chesters (1976); and Yeo (1967). Only minimal research has been carried out on the behavior and fate of herbicide residues that occur in flowing water as a consequence of treating the soil in drained canals or other temporarily dry waterways (Comes et al., 1975, 1976; Frank et al., 1967, 1970; Smith et al., 1975). Except for the work reported by Nelson et al., (1969) and Bartley (1957, 1959), little has been done to determine the roles played by factors other than dilution in the disappearance of phytotoxic chemicals in flowing water. The effects of sediment and other forms of particulate matter have been reported (Coats et al. 1966; Tucker et al., 1967; Adams (1973); Kahn (1974); it is recommended that these latter references be consulted for information on possible effects from sediments and particulates in treated water.

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(c) Methods for determining phytotoxicity of aquatic herbicides to irrigated crops. (1) Introduction. There are no standard protocols for testing the toxicity of herbicide-treated irrigation water. However, some general, acceptable practices should be used as follows: field testing is usually conducted in two steps, small-plot testing using metering devices to control the level of herbicide in irrigation water, and large-scale in-use testing that normally requires an experimental use permit.

(i) Small-plot tests. The small-plot testing should include the following procedures:

(A) Use of replicated plots (preferably at least in triplicate) for each dosage (concentration) tested and for each crop type or variety, as appropriate.

(B) Sufficient dosage ranges to bracket the normal concentrations expected to be present when the product is used at the maximum dosage. Thus, concentrations should include 3x to 4x the maximum expected concentration down to a concentration where no effect on the crops is observable.

(C) Appropriate control or check plots (no herbicide in irrigation water) must be used and must include at least duplicate plots for each crop type.

(D) Irrigation schedules and methods should be as similar as possible to those used in full-scale crop practices. This includes the normal irrigation practices in relation to planting development (growth) of crops.

(E) Metering devices and methods should be accurate enough to maintain the desired concentration within 10% from beginning to end.

(ii) Large-scale tests. Large-scale testing should be made at actual application sites in the field and include the types of crops and irrigation methods that will be used in the areas where the product is to be used. The irrigation schedules must be recorded as well as the amount of water per irrigation.

(2) Reporting effects on crops. In both the large-scale and small plot tests, the condition of the crops must be noted at the various growth stages, including any recovery from initial phytotoxic effects. Appropriate evaluation of crops include crop yield and quality. The yield may be measured in volume, weight or extracted product (e.g. Brix value for viticulture). For seed production, seed viability must be assayed to determine the percent germination during storage life.

(3) Example method references. Initial small pilot procedures such as those used by Bruns and Kelly (1974, 1975) are acceptable for obtaining experimental use permits for full scale tests. Other acceptable methods are found in the references below.

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(d) Efficacy testing methods for aquatic herbicides in standing waters.

(1) Acceptable test plots: The following types of test plots may be used for some types of aquatic plants as described below.

(i) Whole pond treatments. Whole pond treatments have been used for many years and most closely approximate some actual use conditions for aquatic herbicides. First, it is difficult, if not impossible, to obtain comparable replicates. Even adjacent ponds separated by narrow dikes and located on identical soils frequently react in dissimilar manners when treated with herbicides. In addition to making replication difficult, this lack of uniformity makes comparisons between treated and untreated ponds difficult. Usually a comparison of the same pond before and after treatment is more meaningful than comparing a treated pond with an untreated pond, but even this fails to account for population variation with time.

(ii) Open plots. Open plots are treated areas in large bodies of water not enclosed or contained by any artificial means. Open plot treatments have been used successfully under a variety of situations and in some cases closely simulate in-use conditions. A comparison of open plot treatments with whole pond and plastic-enclosure treatments can be found in 3 papers by Walker (1964, 1964, 1965). There are major differences between whole-pond tests and open-plot tests. The first is a problem of dilution of the herbicides by the surrounding untreated waters, which indicate that size of the plot is very important. Although many investigators have used smaller sized plots, open plots should be at least 0.1 hectare to get reliable results. Even with this size plot, border effects might extend to the center of the plots. Data should be taken from the center of the treated areas. For open plots of 0.1 hectare or smaller, standard treatments with pesticides of known effect must be included for comparison with the pesticide being tested. Second, with open plots one must also be cognizant of water movement from currents, tides, and wave action, which might carry the herbicide, or plants, out of the plot boundaries. Because of the plant-drift problem, open plots are not suitable for unattached algae or for free-floating macrophytes. On the other hand, open plots are suitable for emersed and marginal plants, and to a lesser extent for floating-leafed species. One advantage over whole pond treatments is that, with many plots in one body of water, plot-to-plot variation is less.

(iii) Enclosed plots. Enclosed plots are areas in larger bodies of water which are "sealed off" from the rest of the water mass, usually by plastic film. Enclosed plots have been used by various investigators in the past (Gallagher et al., 1968; Walker, 1964, 1965). They offer the smallest, most reproducible type of plots available for use with naturally-occurring plants which have not been disturbed or transplanted and a natural bottom which has been disturbed very little, if at all. They can be quite small because there is no dilution of the herbicide by the surrounding untreated water. This is a distinct advantage over the open-plot method. Also, because of their smaller size [5 x 5 ft (1.52 x 1.52 m) used by Gallagher et al. 1968], visual estimation of herbicidal effectiveness is simpler and possibly more accurate. A major concern with enclosed plots is to assure that the plastic is not permeable to the herbicide and that the herbicide is not adsorbed or absorbed by the particular type of plastic being used. A tight seal at the bottom is also important. The technical difficulties of assuring a tight seal without disturbing the site indicate that this method is less than ideal.

(iv) Artificial pools. Above-ground, plastic-lined or concrete pools offer a convenient system for testing potential aquatic herbicides. The method is described in detail by Lawrence and Blackburn (1962). The same care must be taken as with enclosed plots to assure the herbicide is not adsorbed or absorbed by the plastic liner. The advantage of artificial pools is that a wide number of variables can be studied under semi-controlled conditions, and a wide spectrum of weeds can be tested. Extrapolation of the results of artificial pool tests to natural bodies of water may not be valid. The substrate added to the pool is "artificial" and may not resemble natural pond or lake sediments in either chemical composition or physical structure. Some degree of testing by either the whole pond or open plot method should be done to confirm the results of artifical pool tests.

(2) Application methods. The appropriate method of application depends on formulation, size of plots, label directions for use, and, possibly, rate of application. It is important that the method be described in detail, that the method results in accurately-applied amounts of pesticide, and that the method give reasonably uniform distribution of pesticide throughout the test area. The latter is most important with certain chemicals and with types of vegetation to which spray is directly applied, such as floating mats of algae, free-floating plants, emergent vegetation and floating-leafed species controlled by liquid spray applied to the top surface of leaves.

(3) Evaluation of results. A number of methods have been developed for measuring aquatic plants. One or more of the following should be selected for evaluation of treatments and untreated control plots. The evaluation technique must be appropriate for the type of plant and the nature of the test.

(i) Colorimetric and spectrophotometric techniques. Chlorophyll determinations by spectrophotometric methods are widely used for determining single-celled phytoplankton and small plankton colonies of algae. Any methods detailed by Golterman (1971) and by Weber (1973), such as the technique given in § 92-20(e) may be used. No chlorophyll technique is acceptable for macrophytes, and such techniques are generally unsatisfactory for filamentous algae, since gravimetric techniques are better when sampling is adequate. Visual estimation of water color and/or turbidity is not normally sufficient for measuring degree of control under field conditions. However, when used in conjunction with cell counts, data obtained with the use of a Secchi disc will be acceptable for phytoplankton evaluation.

(ii) Standing crop methods. There are a number of simple quantitative and semi-quantitative sampling techniques available involving collection of plants with rakes, with samplers or by hand (Weber, 1973). These techniques lend themselves to gravimetric determination of standing crop. Dry weight is preferred over wet weight and, due to high surface deposits of inorganic sediments, ash-free dry weight is more reliable than conventional dry weight

(Stanley et al., 1976). An appropriate ash weight technique is given by Weber (1973) in the section on plankton. The greatest disadvantage with gravimetric methods is the difficulty of obtaining an adequate number of samples due to the erratic occurence of aquatic plants. In a dense, uniform stand, 3 to 5 samples may be adequate for a dominant species, but less common kinds of plants may require 30 to 40 samples for an adequate measure of standing crop, and rare species will be impossible to quantify gravimetrically (Livingston et al. 1976). Sampling is particularly difficult for free-floating macrophytes and for floating mats of algae. A gravimetric productivity method has been developed which uses wooden floats to protect duckweeds from drift (Rejmankova, 1973). Similarly, artificial substrates with algal growth could be used to simulate periphyton which have not broken free to form floating mats (Weber, 1973). Consultation with the Agency is advised prior to the use of methods such as these latter two.

(iii) Population counting methods. Direct counts of cells or colonies of plankton may be made microscopically according to the methods outlined by the American Public Health Association, Inc. (1965) or by Weber (1973). On the other extreme, large emersed plants can be counted individually. However, in many cases, prolific asexual reproduction and intricately intertwined population masses make it impossible to distinguish and count individual members of a population.

(iv) Transects and surface cover methods. Transects are among the several terrestrial sampling methods that are adaptable to aquatic plants (Wood, 1963). Both line intersect and transect belt have been used successfully (Kvet and Ondok, 1973; Taylor, 1971). Estimates of surface area coverage in quadrats randomly or systematically located along a transect can be performed more rapidly than standing crop measurements. With the many replicates, which are possible due to the ease of performing the estimate, this technique is as reliable as gravimetric techniques. When casually applied, this method is similar to visual estimates discussed in the following section. The report of evaluations using transects shall include details such as type of transect belt or transect-quadrat, random and systematic selection methods (e.g., transects located randomly within the site and quadrats located systematically based on depth along the transect), and nature of count (at surface of water or surface of substrate).

(v) Visual estimation methods. The majority of tests are evaluated by visually estimating the reduction in vigor and stand density of each species present. When this method is used, more "samples" are required to reduce variation than with more quantitative techniques. Whenever possible, observer bias should be minimized by pooling results of a number of independent observers or by periodically "standardizing" estimates against more reliable quantitative techniques. Visual estimation is not acceptable for phytoplanktons and periphyton populations, for which more precise methods are available. (See American Public Health Association, Inc., 1971; and Methods of Hydrobiology Fresh Water Biology by J. Schwoerbel, 1970.)

(vi) Underwater records. Several of the techniques discussed above can benefit by the use of scuba for evaluation of submersed plants (Timmons, 1970; Wood, 1963). Underwater recording equipment has been developed for performing transect and quadrat counts while completely submersed (Fager et al., 1966), and a quantitative phototechnique has been developed that can be used in clear water (Johnston et al., 1969). Scuba is especially beneficial for careful collection of deeply submersed standing crops (Stanley et al., 1976). If scuba diving observations are used, the report should clearly indicate so.

(vii) Photographic techniques. Aerial photography techniques, already a well developed evaluative technique for terrestrial locations, has been widely used for emersed and marginal aquatic plants (Shima et al., 1976) and can be applied to free-floating and floating-leafed species (Tarnocai and Kristof, 1976). Supported by ground truth, aerial photography can be used for identification (Cowardin and Myers, 1974) and for quantification (Drake, 1976). Complex patterns not distinguishable from the ground can be seen (Wrigler and Horne, 1974). For submersed species penetration of the water column is a serious problem on which progress is being made (Specht et al., 1973). Though relatively inexpensive for evaluating large and/or inaccessible areas, aerial photography is expensive when used for a few small test plots. Aerial photography from a surface-towed ballon may provide some of the advantages of an elevated point-of-view with a cost appropriate for small scale evaluations (Edwards and Brown, 1960). When supported by appropriate ground truth and/or other efficacy data, aerial photography, as well as conventional photography, is an acceptable evaluation technique.

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(e) Pure-culture technique for evaluating algicides. This procedure is a modified version of a method first described by Fitzgerald and Faust in 1963. (See also Fitzgerald, 1962, and 1964). This modified procedure has been used at the Agency's Chemical and Biological Investigations Branch Laboratories (Benefits and Field Studies Division) for several years to help determine effectiveness of algicides as part of the Agency pesticide enforcement activities. These modified procedures are published in the Manual of Biological Testing Methods for Pesticides and Devices.

A method of test for evaluating algicides using pure-culture techniques.

1. Scope 1.1 Products intended for the control of algae in swimming pools, industrial cooling water systems, and other water systems should be shown to be effective for the purpose claimed. This pure-culture test would determine the effectiveness of a chemical formulation to control standard algae species, and also show the algicidal vs. algistatic properties of the formulation being tested.

2. Test Organisms

2.1 Chlorella pyrenoidosa. (Wis. 2005) or Chlorella pyrenoidosa Chick No. 26 (Obtained from Dr. Richard C. Starr, Culture Collection of Algae, Department of Botany, University of Texas, Austin, Texas. 78712.

2.2 Phormidium inundatum and Phormidium retzii, should be included in the test as representative algal species found in swimming pools. Other algae may also be included if deemed necessary. For culture media for Phormidium species, see Hughes, et al. (1958). The concentrations of stock cultures must be adjusted in such a way that test inoculum will be reproducible and equivalent to a standard of Chlorella. This may be accomplished by adjusting Phormidium inocula to a predetermined Chlorella inoculum optical density. (See Fitzgerald, 1964).

2.3 Modifications for use in developing presumptive efficacy data for industrial cooling system products: Use the following algae: *Chlorella pyrenoidosa*, *Euglena gracilis*, *Scenedesmus obliquus*. Obtain from the addresses in section 2.1 as follows: *C. pyrenoidosa* - Fitzgerald or Starr; *E. gracilis* and *S. obliquus* Starr.

3. Culture Medium

3.1 Allen's Medium (Difco Algae Broth may be used) for *Chlorella pyrenoidosa*.

Distilled water	1000 ml
NH_4Cl	50 mg
$NaNO_3$	1000 mg
K_2HPO_4	250 mg
$MgSO_4 \cdot 7H_2O$	513 mg
$CaCl_2 \cdot 2H_2O$	66 mg
$FeCl_3$	3 mg

3.2 Stock cultures are maintained on Allen's medium (or Difco Bacto "Algae Culture Agar") as 1.5 percent agar slant cultures in tubes.

3.3 Stock cultures stored at 22 to 24°C with 16 hrs of cool white fluorescent light per day, 4306 lx (400 ft. candles) initially and then removed to low light conditions.

3.4 Media for algae used for industrial cooling system assays:

3.4.1 Hunter's Medium for *Scenedesmus obliquus*:

	Amount per liter:
KH_2PO_4	0.8 g
$MgSO_4 \cdot 7H20$	1.9 g
L-glutamic acid	10.0 g
DL-malic acid	4.0 g
$CaCO_3$	0.4 g
$FeCl_3 \cdot 6H_2O$	0.0167 g
Thiamine HCl	2.0 mg
$(NH_4)_2HPO_4$	0.4 g
Vitamin B_{12}	0.4 ug
$ZnSO_4 \cdot 7H_2O$	176.0 mg
$MnSO_4 \cdot 4H_2O$	160.0 mg
$Na_2MoO_4 \cdot 2H_2O$	40.0 mg
$CoCl_2 \cdot 6H_2O$	3.2 mg
$CuSO_4 \cdot 5H_2O$	1.6 mg
H_3BO_3	1.2 mg
NaI	47.0 ug

3.4.2 Bristol's Medium for *Euglena* (or Difco Bacto *Euglena* Broth): Bristol's medium, supplemented (per liter)

$NaNO_3$	505 mg
$CaCl_2$	50 mg
$MgSO_4 \cdot 7H_2O$	150 mg
K_2HPO_4	150 mg
KH_2PO_4	350 mg
NaCl	50 mg
$MnSO_4 \cdot 4H_2O$	300 ug
$FeCl_3$	1 ug
$ZnSO_4$	200 ug
H_3BO_3	200 ug
$CuSO_4 \cdot 5H_2O$	6 ug
yeast extract	1 g
proteose peptone	1 g
glucose	15 g

4. Apparatus

4.1 Glassware: 50-ml Erlenmeyer flasks with cotton plugs for each species. Suitable pipettes for addition of chemical formulation to test flasks.

4.2 Bacteriological loop (0.01 ml) for making subcultures.

4.3 Controlled-environment chamber for holding temperature at 22-24°C. Chamber equipped with cool white fluorescent lights giving 4306 lx. Lights are regulated with time clock to operate for 16 hours per day.

4.4 Automatic pipetter (facilitates preparation of large numbers of test flasks).

4.5 Steam sterilizer.

4.6 Spencer Bright Line Hemacytometer (or equivalent) for counting algae.

4.7 Bunsen burner for sterilizing bacteriological loops.

4.8 Laboratory microscope for use in making algae counts.

5. Procedure

5.1 Transfer algae from stock agar cultures (3.2) to media and hold at 22-24°C under 16 hours fluorescent light, 4306 lx in suitable containers for up to 3 weeks to inoculate test flasks.

5.2 Place 30 ml of medium into each 50-ml Erlenmeyer flask. This may be accomplished quickly with the use of an automatic pipetter. Plug flasks with cotton and sterilized at 103 kpa (15 psi) for 20 min. After they have cooled to room temperature the flasks are inoculated with *C. pyrenoidosa*.

Inoculate from an actively growing liquid culture (5.1) at a concentration to give an initial cell count of 300,000 cells/ml in each test flask. No more than 0.3 ml aliquot should be taken from the liquid stock culture for addition to each test flask in order to give the desired algae concentration of 300,000 cells per ml. Centrifugation of liquid stock culture or other means may be necessary in order to obtain desired concentrations of algae cells.

5.3 Add chemical formulation at each rate to be tested to 10 flasks. Ten inoculated flasks are used as untreated controls for each species.

5.4 To determine algicidal vs. algistatic properties of the chemical formulation, 0.01 ml aliquots, using a 0.01 ml bacteriological loop, are aseptically transferred from the primary cultures after 2 days of incubation, to 10 additional 50 ml Erlenmeyer flasks, each containing 30 ml of sterile growth medium. Subcultures are also made of controls. The subcultures are kept under the same environmental conditions as the primary cultures for three weeks.

5.5 Flasks should be continuously shaken during test (approximately 100 oscillations per minute).

6. Determination of results.

6.1 Evaluation: Record visual observations weekly for duration of the test. Observations are made of both the primary cultures and subcultures. A rating scale may be used to help determine actual percent control by the chemical formulation such as, 0-5, "0", being no visible growth, and "5" very heavy growth. Other means, such as colorimetric methods, may be employed to evaluate results of the test.

6.2 Interpretation: For a product to be satisfactory it must provide at least 70% control of algae growth in the primary cultures for 3 weeks. When using a rating system, percent control is obtained by subtracting the average rating for the 10 treated flasks (RT) from the average rating of the 10 untreated control flasks (RC), then dividing by the average rating figure of the untreated flasks (RC), multiplied by 100. [Percent control = 100 (RC - RT)/RC.] In order for an algicidal product to claim "kill" or "destroy" algae on the label, there must be no visible sign of algae growth in subcultures after 3 weeks.

References.

Chemical and Biological Investigations Laboratories. 1978. Manual of Biological Testing Methods for Pesticides and Devices. Schneider, B.A., and A. J. Culver, Jr. ed. U.S. Environ. Prot. Agency, Benefits and Field Studies Div., Beltsville, Md.

Fitzgerald, George P. 1962. Bioassay for algicidal chemicals in swimming pools. Water and Sewage Works 109:361-363.

Fitzgerald, George P. 1964. Factors in the testing and application of algicides. Appl. Microbiol. 12:247-253.

Fitzgerald, G.P., and L. Faust. 1963. Bioassay for algicidal vs. algistatic chemicals. Water and Sewage Works 110:296-298.

(f) Method for evaluating algicides in simulated swimming pools

1. Scope

1.1 Products intended for use to control, prevent or inhibit algae growth in swimming pools should be effective. This test method is designed to determine effectiveness of candidate products to control algae in simulated swimming pools.

2. Inoculum

2.1 Test Algae - The genera of algae infesting swimming pools may vary greatly from pool to pool. The species may differ geographically, in response to climate, water hardness, pH, and other factors. Since this method is not a pure culture technique, it would be very difficult to maintain a predominant flora in a given pool situation. Therefore, the choice of test organisms will be wide. However, representative isolates of the following algae should be included in the inoculum for each test pool:

Chlorella pyrenoidosa
Phormidium inundatum
Phormidium retzii

These genera have been found in swimming pool water. Pure cultures of these algae can be obtained by isolation from sources in every location where needed, or from commercial or institutional culture collections, such as American Type Culture Collection, Rockville, Md. 20852, and the Culture Collection of Algae, University of Texas, Austin, Tex. 78712. They should be maintained in synthetic nutrient medium. (See 3. Synthetic algal nutrient medium.)

2.2 Maintenance of Stock Cultures.

2.2.1 Medium - see 3.

2.2.2 Incubation Conditions - 24°C ± 1, cultures exposed to 16 h photoperiod, 3229 lx (300 ft candles), cool white fluorescent light.

2.2.3 Age of cultures - Two to three weeks with good growth evident.

2.3 Preparation of inoculum for test.

2.3.1 Culture units - Prepare one flask of medium (see 3) for each algae times each pool in the test. Use 1000 ml culture flasks containing 500 ml of medium. Autoclave 20 min at 103 kpa (15 psi) and allow to cool prior to inoculation.

2.3.2 Inoculate sufficient culture units, using aseptic methods, to supply the test pools.

2.3.3 Incubate as in 2.2.2 and 2.2.3.

3. Synthetic algal nutrient medium

3.1 Final concentration of nutrients (1).

3.1.1 Macronutrients - The following salts, Biological or Reagent grade, in mg/l of glass-distilled water:

Compound	Concentration (mg/l)
$NaNO_3$	25.5
K_2HPO_4	1.044
$MgCl_2$	5.7
$MgSO_4 \cdot 7H_2O$	14.7
$CaCl_2 \cdot 2H_2O$	4.41
$NaHCO_3$	15.0

3.1.2 Micronutrients - The following salts, Biological or Reagent grade, in ug/l of glass-distilled water:

Compound	Concentration (ug/l)
H_3BO_3	185.52
$MnCl_2$	264.264
$ZnCl_2$	32.709
$CoCl_2$	0.78
$CuCl_2$	0.009
$Na_2MoO_4 \cdot 2H_2O$	7.26
$FeCl_3$	96.0
$Na_2EDTA \cdot 2H_2O$	300.0

3.2 Stock solutions.

3.2.1 Macronutrients - Stock solutions of individual salts may be made up in 1000 times the final concentration.

3.2.2 Micronutrients - The trace metals, $FeCl_3$, and EDTA are combined in a single stock mix at 1000 times the final concentration.

3.3 Preparation of medium

3.3.1 Combination of stock solutions - One ml of each of the stock solutions (3.2.1 and 3.2.2) is added to glass-distilled water to give a final volume of one liter.

4. Apparatus

4.1 Test pools, 1.22 by 0.61 m (4 by 4 by 2 ft) deep are constructed of 19 mm (3/4 in.) plywood, framed with 50.8 by 101.6 mm (2 by 4 in.) lumber. Vinyl or polyethylene liners [1] are fitted into the supporting wood box. The corners are either heat-sealed to make a clean joint or the excess plastic is folded under, the watertight integrity being carefully preserved. Pools of this size hold 908 liters (240 U.S. gal). Each pool is equipped with a pump, in-line filter, and heater. The heater is controlled by thermoregulator, adjusted to 24°C. The pump is a submersible Little Giant Model 2E38N, 110V 60 cycles, or equivalent. The filter is a cellulose cartridge type such as Teel (W.W. Grainger) Stock No. 1P635 equipped with filter cartridge Stock No. 1P753. Filter elements of different manufacture but of similar composition may be substituted.

5. Procedure of test

5.1 Preparation of Pools - Fill with tap water to 25 mm (1 in.) from top. Allow to equilibrate for 24 hr.

5.2 Replications - Each treatment should have a minimum of two replicates. If this is not feasible because of space limitations, two consecutive tests must be conducted.

5.3 Nutrients, temperature, and light - Add to each pool 100g of Ra-Pid-Gro Soluble Plant Food (Ra-Pid-Gro Corporation, Dansville, N.Y. 14437) or equivalent, at the time of filling. After the 24 hr. equilibration period adjust the temperature to 24°C ± 1 with the circulating pump running and the filter in place. Maintain 16 h photoperiod, 3229 lx, cool white fluorscent light over all pools, or daylight, if available.

5.4 Inoculation - inoculate each pool with one flask (2.3.1) of each algae being used in the test. Remove all attached algae from flask with a brush or other means and wash into pool with pool water.

5.5 Chlorine standard - Maintain at least one pool as a chlorine standard with no additional algicidal treatment added. For pools stabilized with isocyanuric acid (trihydroxytriazinetrione), maintain chlorine residual at 1.0 to 1.5 ppm continuously. For non-stabilized pools, chlorine residual should be incorporated in all tests at the rate of 0.6 to 1.0 ppm. Metering devices may be used, provided they will accurately maintain the desired chlorine level.

5.6 Water flow rate - Adjust flow rate to provide a complete filtration cycle in about 6 h. For a 908 liter pool, this would require a pumping rate of 151 l/hr (40 gal/hr), or 2.5 l/min (0.66 gal/min).

5.7 Adjust pH daily and maintain between 7.2 to 7.6 during the period of the test. To raise pH, add sufficient soda ash (sodium carbonate) to bring the pH to the above range in each pool. These chemicals should be placed in a plastic strainer and allowed to dissolve under the inlet water flow. Algal growth will result in an increase in the pH. This increase must be counteracted with the proper treatment above.

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[1] Liners may be obtained from Plastimayd Corp., 2204 S.E., 7th Ave., Portland, Oregon 97214

5.8 Treatment - A minimum of two replications per treatment should be included in each test. Apply product at initial and maintenance doses. Maintain 1 or more untreated control tanks and one chlorine standard tank.

5.9 Length of test period - Maintain conditions as in 5.3 above for a minimum of four weeks.

6.0 Reporting Results - Examine tanks weekly. Rate amount of algal growth using a scale such as:

Numerical rating	Status of algal growth
0	No apparent growth
1	Barely visible attached mat
2	Clearly visible mat, slight bloom
3	Well defined mat, moderate bloom, bottom still visible
4	Walls and bottom covered by mat, bottom barely visible
5	Heavy bloom, loss of water transparency, heavy attached or free-floating mats

6.1 Products which give a final average reading greater than that of the chlorine standard should be considered unsatisfactory for use in swimming pool service. In addition to estimation of algae by visual inspection, swimming pool algicide effectiveness should be evaluated by determining the remaining inoculated algal species in pool water samples taken at bi-weekly intervals throughout the test period. Other valid means of evaluation may be used when determining the results of the test.

References.

(1) Chemical and Biological Investigations Branch Laboratories. 1978. Manual of Biological Testing Methods for Pesticides and Devices. McCann, J., and A.J. Culver, Jr., eds. U.S. Environ. Prot. Agency, ARC-East, Beltsville, Md. 20705.

(2) National Eutrophication Research Program, Environmental Protection Agency, August 1971, "Algal Assay Procedure, Bottle Test", U.S. Government Printing Office, 1972, Region No. 10. Pp. 11-12.

(3) "Standard Methods for Water and Wastewater", APHA, 13th Ed., 1971. Pp. 123-124.

Series 93: EFFICACY OF FUNGICIDES AND NEMATICIDES

§ 93-1 General considerations.

For complete guidance on the performance of fungicides and nematicides, the information contained in §§ 90-1 Overview and 90-2 General considerations must be considered in conjunction with the information in §§ 93-2 through -15.

§ 93-2 Definitions.

(a) The term "fungicide" means any substance or mixture of substances intended for preventing or inhibiting the growth of, or destroying any fungus (or any plant disease agent such as bacteria, mycoplasma, and virus) declared to be a pest under § 162.14 of the FIFRA Sec. 3 regulations, except those fungal control agents described in § 92-4 and -5 as "microbiocides", those defined in § 91-1 as "antimicrobial agents", and those subject to the provisions of the Federal Food, Drug, and Cosmetic Act, as amended (21 USC § 301 *et seq*.)

(b) The term "nematicide" means any substance or mixture of substances intended for preventing or inhibiting the multiplication or establishment of, preventing or mitigating the adverse effects of, repelling, or destroying any member of the Class *Nematoda* of the Phylum *Nemathelminthes* declared to be a pest under § 162.14 of the FIFRA Sec. 3 regulations.

§ 93-3 Suggested performance standards: acceptable levels of pest control.

(a) Due to the diverse factors involved in the control of plant diseases or nematodes, the degree of control that is acceptable or attainable under certain use conditions for a particular pest/site combination may not be acceptable under other conditions. The acceptable level of pest control for fungicides and nematicides will vary for each pest/site combination, user group, end use of treated site, and the degree of control intended to be claimed on labeling (e.g., controls, aids in control, prevents, suppresses). In general, the acceptable level of control for a given use will be that which the ultimate user of the pesticide would find acceptable. [See Subdivision H, §§ 103- for more information on fungicide labeling].

(b) For claims that a product "controls" or "prevents" plant disease or nematode pests, the product should generally provide, under moderate to severe pest pressure, at least 70% control of the pest organisms or their symptoms (compared with untreated controls) over the period in which control is intended. For control of nonagricultural fungal pest problems, the product should generally provide complete control of the pest problem for the expected storage period or service-life of the substance to be protected.

(c) Under certain circumstances a level of effectiveness less than that which is considered optimum or complete may be claimed and be appropriate. Lesser claims, such as "aids in control" or "suppresses", may be made if less than 70% control of plant diseases or nematodes is obtained, provided that a measurable benefit (e.g., increased quality or quantity of crop yield) can be demonstrated. Such benefits are usually only attained under certain circumstances which must be determined from the test results. These use limitations must be stated on the product label. For example, the label might state that the suppression of a given pest may only provide a benefit when low pest populations exist.

§ 93-4 Products for use against above-ground plant pests.

(a) Control of plant diseases that attack above-ground plant parts should be evaluated in tests conducted under in-use conditions which utilize comparisons of untreated and treated plants. Separate tests should be conducted using each intended type of application equipment, application rate and schedule, type of spray (i.e., full coverage, low volume, or aerial), type of control program (i.e., preventive or curative), and cultural practice (e.g., greenhouse, field, irrigated, nonirrigated). Field tests should be conducted at a sufficient number of geographic locations so as to include the expected range of rainfall, relative humidity, air temperature, frequency and type of irrigation, planting dates, crop cultivars (varieties), and other pertinent factors (appropriate for the pest/crop combination) associated with the intended geographic area of use which may affect product efficacy.

(b) The control of two or more concurrent diseases of the same crop may be evaluated in the same test, provided that the disease symptoms are readily distinguishable and allow for separate evaluations. In cases where different stages of the same disease attack different plant parts, the stage(s) intended to be controlled must be reported and supported by the appropriate pest control data. A considerable number of disease assessment criteria are available, e.g., number of lesions per leaf, number of affected plants per specified row length. Since the precision and reliability of each criterion will vary with the pest/crop combination under study, the investigator should carefully select the best criteria for detecting valid treatment differences in each test. In addition to the criteria used, the determination of proper time and number of disease evaluations is also important. Such observations are generally made at the time of initial application and at periodic intervals throughout the growing season. When repeat applications are intended, it is advisable to make disease observations on each application date to determine appropriateness of the time interval between applications.

(c) Test reports, when applicable, should include detailed information on the afore-mentioned test and evaluation variables, as well as the following: the disease level at the time of initial application in curative tests; the disease level and date when disease first appeared in test plots, in preventive tests; a complete description of any rating system used (i.e., criteria for each rating value); the nature and degree of phytotoxicity which may result from use of the product at or above the intended label dosage rates; and the nature and extent of any noticeable spray deposits on plant foliage, fruit, or flowers.

§ 93-5 Products for use against soil-borne plant pests.

(a) Tests for fungicides and nematicides applied to soil should be designed to determine safety to crops grown on the target site, and to determine effectiveness against target soil-inhabiting organisms present in soils of different textures, moisture and pH levels, organic and mineral compositions, and under a range of climatic conditions common to potential target sites.

(b) All specialized application equipment and techniques should be adequately described.

(c) Evidence of pest control or repression of diagnostic symptoms should be provided. Data which show beneficial effects such as plant growth or yield increase are unacceptable as the sole proof of product effectiveness; however, such data are necessary to demonstrate product usefulness. Depending upon the plant pathogen or nematode and crop involved, important factors to be measured in assessing pest control include, but are not limited to, one or more of the following:

(1) Crop emergence,

(2) Pest mortality or survival,

(3) Crop stand,

(4) Crop lodging,

(5) Crop root injury,

(6) Disease-free or marketable crop yield,

(7) Crop yield or growth response, and

(8) Pest and/or symptom expression ratings.

(d) When a general nematode claim on product labeling is intended, effectiveness data should be developed to demonstrate control of at least one species of root-knot, cyst, migratory endoparasitic nematode, and ectoparastic nematode of importance on the crop(s) or crop planting site(s) to be treated. However, the test results should demonstrate that the product will control all the important parasitic nematodes associated with a given site; otherwise claims should be limited to the specific type(s) that can be controlled. Nematode control may be effectively evaluated by comparisons of treated and untreated plants, plant parts, or soil using one or more of the following criteria:

(1) The degree of galling on roots, or root-knot index (rootknot nematodes).

(2) Number of nematodes per unit volume or weight of soil (any parasitic nematodes).

(3) Number of nematodes per unit measure of roots or other plant parts (lesion and other endoparasitic nematodes).

(4) Number of cysts containing viable eggs and/or larvae per unit measure of soil and/or roots (cyst nematodes).

(e) The overall results should statistically demonstrate that nematode control achieves a desired quality, quantity, or other measurable crop benefit. Pest population comparisons should be made between treated and untreated plots before or at treatment and at appropriate intervals thereafter. Proper sampling times will vary depending upon the nature of the chemical and type of crop and nematode. For example, final population counts obtained at the end of the growing season in treated areas often exceed those in untreated areas, particularly when early season control results in increased plant growth; therefore such counts are unreliable measurements of control. See § 93-30 Item 15 for additional guidance on conducting nematicide tests.

§ 93-6 Products for post harvest use on fruits and vegetables.

(a) The evaluation of pre harvest or post harvest fruit and vegetable treatments for control of post harvest fungal or bacterial disease needs replicated tests on lots which are of sufficient size to represent commercial size storage containers.

(b) Measurement of disease levels should be taken following actual or simulated packing, storage, and transportation procedures normally encountered with the particular crop under study.

(c) Whether crops are treated before or during storage, disease measurements should be obtained and recorded periodically during storage. For each replicate, these measurements ordinarily should be expressed in terms of the percentage of diseased produce.

(d) Usually, separate tests should be conducted on each specific fruit or vegetable and on each organism to be controlled. However, if two or more readily distinguishable diseases are present on the same host simultaneously, their control may be evaluated in the same test. Disease control data on naturally- or artifically-inoculated produce is acceptable.

(e) The product should be tested with each type of equipment (bin-type dumpers, tank washers, spray washers, brush cleaners, hydro-coolers, and similar devices) named in the directions for use.

§ 93-7 Products for use as grain preservatives.

(a) Fungicides intended for use in the preservation of stored, high-moisture grain should be evaluated in both laboratory tests and in-use site tests. Tests should include treatments over a range of dosages and grain moisture levels. High-moisture grain generally implies a moisture content of at least 15 percent. Product effectiveness evaluations in both laboratory tests and in-use site tests are necessary and should include comparisons of treated and untreated grain samples. These evaluations are based on visible mold growth or actual microorganism counts and auxiliary criteria, such as temperature rise and respiration rate of stored grain.

(b) Laboratory tests should be designed to determine the presumptive dosage rate(s) necessary to control fungi in or on naturally-infested or artificially-infested grain (usually inoculated with species of *Aspergillus*, *Alternaria*, *Fusarium*, and *Penicillium*). When high moisture grain is not available, naturally dried grain reconstituted to the desired moisture level may be used. Test samples may consist of one quart (946 ml) to 25 gallon (94.6 l) volumes of grain in partially closed glass or plastic containers. Insulated containers, such as thermos bottles, are necessary for tests with small samples where ambient storage temperatures are not controlled. Test variables should include, but are not limited to: treatment rates, grain moisture levels, kinds of grain, and spoilage organisms.

(c) Once the preliminary efficacy of the product is established in the laboratory, additional tests must be conducted under actual in-use storage conditions. If treated grain is to be stored in enclosed outdoor structures (e.g., bins, sheds, or silos) such tests should be performed in either a structure of this type having a capacity of at least 100 bushels (35.2 hl), or in small-scale unexposed outdoor structures (i.e., structures contained within a shed or similar enclosure) with a capacity of at least 10 bushels (3.5 hl). If other types of storage are intended, the product should be tested under those storage conditions. Tests should contain a minimum of two (preferably more) replicates for each treatment. The fungicide should be tested at the proposed application rate(s) in several geographic locations where grain is commercially produced and stored. Since stored high-moisture grain will spoil rapidly, testing of the product on grains under in-use storage conditions should begin within a few hours of harvest. The duration of testing must correspond with claims to be made on the labeling (generally 6 to 12 months). Applicable effectiveness evaluations should be recorded periodically during storage.

(d) Depending upon the end-use of treated grain, additional studies may be needed. If treated grain is to be used in animal feeds, appropriate feeding studies should be performed, preferably with poultry and cattle, to demonstrate the acceptability of treated feeds to animals. If grain is to be used for planting, phytotoxicity tests must be conducted under field conditions. [For guidance on phytotoxicity tests, see also § 93-8(b) of this subdivision and Subdivision J of these guidelines.] In cases where treated grain may be used for human food or processed into products for human consumption, an evaluation of the acceptability of flavor, milling and baking qualities, fermentation properties, or other important characteristics should be conducted.

§ 93-8 Products for use as seed treatments.

(a) Seed-treatment products intended for use in the control of post-planting diseases of seed, seedlings, or mature plants should be evaluated in laboratory, or greenhouse and field tests, utilizing seed lots and/or soil known to contain high levels of specific disease-producing fungi. Product performance under field conditions should be demonstrated utilizing the intended types of application equipment. Since treated seed may be stored for a number of months before planting, data should be developed which demonstrate that the product will be effective and nonphytotoxic after storing the treated seed for the maximum storage time generally associated with the type of seed intended to be treated. If such storage data are not obtained, the time of application on labeling should be limited to the maximum time period between treatment and planting that is supported by the test results.

(b) Seed decay and seedling blight disease control can be evaluated in laboratory and greenhouse tests, provided that the results of such tests are supported by a limited number of field tests. The field tests need not have a wide geographical distribution to support claims for seed decay and seedling blight control. In laboratory and greenhouse tests, moderate to severe disease incidences can be assured by techniques such as initially slowing germination with reduced temperature or by increasing inoculum levels of specific pathogens. Results are usually expressed as the percent emergence and percent survival of seedlings on two or more observation dates. Noticeable delays in seed germination or seedling emergence observed under field conditions will require that such tests be continued for the duration of the growing season to determine effects on yield.

(c) Products intended for control of soilborne and/or seedborne diseases such as blights, rusts, and smuts, which primarily affect mature plants, must be tested under different soil and climatic field conditions in areas where the crop is likely to be grown. Evaluations should be made of emergence, stand, percent disease, and yields.

(d) In any seed treatment field tests, well-replicated small plots should be used with seeding rates and plant spacings comparable to general cultural practices. The number of replications and the size of each plot must be consistent with the known variability of soil, the character of the disease, and the anticipated disease incidence. Tests in any one location should contain a minimum of 4 to 6 replicates per treatment. Replicates generally consist of one or two rows of 50 to 100 plants for cotton, peanuts, and other large-seeded crops, or single rod-row plots for grains and other small-seeded crops. Larger plots will be needed if samples are to be collected for residue analysis, or if grower planting or harvesting equipment is to be used, or if low disease incidence is expected. Tests should be designed to span several planting dates and locations over a range of conditions, and for evaluation of several of the principal varieties of each crop for phytotoxic response. It is desirable to have seed treatment materials evaluated in regional trials, such as those conducted by the Regional Cotton Seed Treatment Committee of the Cotton Disease Council. (For further information, contact: National Cotton Council, P.O. Box 12285, Memphis, Tennessee 38112).

§ 93-9 Products for use on ornamental and flowering plants.

Final-stage testing of fungicides and nematicides for use on ornamental plants must be conducted in replicated field or greenhouse plots, depending on the intended use of the product. Separate greenhouse and field tests for effectiveness and phytotoxicity are required if both use areas are intended. In the case of florist crops, acceptable levels of phytotoxicity and visible pesticide spray deposits are generally lower than those tolerated for other ornamental crop uses. The commercial grower's assessment of the product under actual use conditions is therefore strongly recommended for uses on florist crops.

§ 93-10 Products for use on bulbs, corms, and tubers.

Tests to control storage rots in ornamental bulbs, corms, and tubers should be conducted with 5 or more replications of 100 or more bulbs. Apparently healthly bulbs selected from heavily diseased stock and/or artificially inoculated healthy bulbs should be used for disease control tests; bulbs for phytotoxicity tests (see also Subdivision J) should be selected from stocks that are as disease-free as possible. Disease measurements are usually obtained during and at the conclusion of the storage season. However, testing should continue throughout a complete growing cycle and an additional storage and growing cycle (or forcing cycle) to determine the residual activity and phytotoxicity of the product. Effectiveness evaluations are usually based on comparisons of the final yield (weight, size, and number) of healthy and diseased bulbs within and between treatments. Separate tests should be conducted for each plant species and each pathogen. However, if two or more readily-distinguishable diseases are present on the same host simultaneously, their control may be evaluated in the same test.

§ 93-11 Products for use on trees.

(a) The number of mature trees of a given species to be included in a test depends upon the nature of the disease and the method of treatment. When specimens are the same size and vigor and are growing on similar sites (e.g., similar as to stand density, cultural conditions, soil type, and available water and drainage) or when transmission of disease is dependent upon climatic factors, few trees (i.e., less than 100) per treatment are needed. However, a minimum of 100 single-tree replicates per treatment would ordinarily be required to produce statistically sound data when any of the above conditions are variable, or when disease transmission is dependent upon the presence of insect vectors or root grafts. Since securing enough trees within a small geographic or climatic area (e.g., one or more contiguous counties) is often impossible, the tests must be carefully designed to yield valid test results. When such testing conditions exist, pairing should be used for making treatment comparisons (i.e., each treated tree is paired with an untreated tree which is in close proximity and is similar in size and degree of disease development at the time of treatment). In cases where the disease is widely distributed, separate tests from the major geographic and climatic areas are necessary, or else labeling claims would be limited to the specific area(s) of test.

(b) Depending on the nature and extent of the disease, replicated blocks of 9 to 100 sq ft (0.84 to 9.3 sq m) each should be used for tree seed and seedling bed treatments. Separate tests should be conducted for each crop production practice (greenhouse, field, container) where treatment is intended. One hundred single-tree replicates, or replicated blocks of 4 to 20 saplings each, may be necessary to produce statistically sound data for trees grown in nurseries.

§ 93-12 Products for use on turf.

(a) The size of plots and number of replicates for tests on lawns and other fine turf areas depends on disease uniformity and severity in the test area, and the type and size of application equipment. The control of two or more diseases can be evaluated in the same test when their symptoms are readily distinguishable.

(b) To support a general claim for use on turf, lawns, or grasses (non-grazing areas), the data should demonstrate effectiveness against the same pathogen on at least two cool season grasses (e.g., bentgrass, bluegrass, fescue) and at least two warm season grasses (e.g., Bermudagrass, Zoysia, St. Augustinegrass). Additional tests should be conducted on other representative grasses likely to be treated to determine whether or not the product is phytotoxic at dosages equal to and greater (generally 2-4x) than label rates. (See also Subdivision J.) Claims for use on specific types of grass will be acceptable for those types for which adequate test data are developed. Products intented to contol diseases of Dichondra and other non-grass lawn plants should be tested on the intended hosts under their normal field conditions.

§ 93-13 Treatments for wood and wood products.

(a) Products which are used to control fungal rot and decay of raw and finished wood, or to control surface molds and fungal staining of fresh-cut lumber, require registration. These products are commonly applied to logs, lumber, plywood, millworked products, poles, posts, pilings, timber, wood chips, sawdust, and other wood-based material. Note: Water repellents and sealers which bear claims for wood preservation need not be registered, but the label must clearly indicate that the product has no direct effect on pests and that effectiveness is attributable only to the water repellent or sealant properties.

(b) Wood treatment products can be applied by several pressure and nonpressure methods of application which provide a wide range of preservative penetration and retention levels. Each application method is intended to afford protection for various wood dimensions, periods of time, extremes of exposure, and occasionally wood species. Farm and home applications for treating fence posts and other wood include two or more flowing brush or spray coats, 3-minute dips, hot and cold baths, or 12- to 48-hour soaks. The typical variety of commercial application methods include:

(1) Applications to timber and lumber using pressure methods of impregnation (e.g., "full-cell" or "empty-cell" processes); occasionally double diffusion techniques are used.

(2) Treatment of sawmill lumber, poles, and pilings by passing the freshly cut pieces through pressure tanks or spray hoods.

(3) Treatment of stored chips and sawdust by spray applications as they move on a conveyor belt to the storage area.

(4) Treatment of window sash, frames, and other millwork, either before or after assembly, using diptank or vacuum process methods.

(5) Treatment of standing poles using diffusion processes, which may involve grease, liquid, or impregnated bandage surface applications, liquids injected into poles, or liquids placed in holes bored in poles.

(c) Effectiveness data should generally be developed under field conditions, utilizing wood products representative of those intended to be treated, in several geographic areas representative of the exposure extremes under which the treated wood will be used. The test program and individual test procedures must be designed to consider the pertinent factors associated with the intended use pattern(s) such as: wood species; physical dimensions of wood products; soil moisture and temperature exposure; rain, humidity, solar radiation, and air temperature exposure; surface roughness of wood, wood quality; effect of water repellents or sealants on efficacy of formulation; duration of pest control; soil contact exposure; proportion of heartwood and sapwood; wood moisture content at time of treatment; heartwood penetrability; and protection afforded wood joints, bolt or nail holes, and other difficult to treat surfaces. See § 93-30 Items 9-11 for suggested procedures for evaluating certain wood treatment uses. Note: Once adequate efficacy data have been obtained to support one method of application for a given wood treatment product, data which demonstrate comparable penetration and retention levels may be used to support other methods of application to the same wood products.

§ 93-14 Treatments for industrial materials and equipment.

(a) Industrial fungicides are used to prevent fungal deterioration, disfigurement, or functional impairment of a great variety of products and equipment such as broom corn, cellulose sponges, coatings, cordage, drilling muds, dye baths, emulsions, fabrics, fuels, latex, leather, metalworking fluids, paper, paperboard, plastics, resins, rubber, and transportation and storage equipment. Test procedures for evaluating pest control will vary with the material or equipment to be protected, nature of the pest problem, fungicide formulation, and method of application. Such tests must be conducted under actual or simulated use conditions which reflect the most severe pest problem situation(s) likely to be encountered. The test program/individual test procedures should be designed to consider the pertinent factors associated with the intended use patterns(s), such as: selection of an appropriate range of representative test substrates for each type of substrate intended to be treated; presence of appropriate level(s) and type(s) of fungal pest organisms; use of highly susceptible test substrates (when appropriate, some evaluations should also include low and moderately susceptible substrates); conditions conducive to maximum pest growth/damage; duration and level of pest control needed by intended users; conditions most likely to adversely affect fungicide efficacy (e.g., temperature, humidity, pH, solar radiation, leaching); the most appropriate means of expressing the dosage rate; and the most meaningful measurements to be used in expressing pest numbers (or growth) and, when appropriate, pest damage. When laboratory tests (other than those cited in the § 93-30) are being considered as the sole means of demonstrating product efficacy, it is recommended that the proposed methods be submitted to the Agency for review prior to initiating such tests. Any requests for review of test methods must be accompanied by documentation which demonstrates that the proposed method is reliable (e.g., data from studies designed to compare results from the laboratory test with in-use tests). When new or unusual pest problems are involved, background information on the nature and extent of the pest problem should also be submitted. See § 93-30 Items 7, 8, 12, 13, and 14 for suggested methods to evaluate some of these uses.

(b) Information should also be presented which demonstrates whether or not undesirable changes in physical characteristics (e.g., unwanted discoloration, tenderizing of fabrics) and interference with processes or reactions will occur when fungicides are used as directed, under normal use conditions. If the effectiveness tests are not adequate to make these evaluations, then additional tests designed for this purpose must be conducted.

§ 93-15 Products for control of mold and mildew on surfaces.

(a) The efficacy of products intended to prevent the growth of mold and mildew is greatly affected by the type of surface to which the products are applied. Test methods for representative surfaces are included in § 93-30 Items 1-4. If the surfaces to be treated, or the methods of application, or the organisms to be controlled by the product, are not the same as those indicated in the method, the method should be modified to reflect these differences. Modifications should also be made so that the method will more clearly reflect actual in-use conditions (including any specialized use situations). For example, tests for products with fungistatic claims intended for use in shower stalls should include test data to indicate whether leaching will alter the efficacy of the product. Any modifications of test methods must be reported along with justification for the change submitted.

(b) The efficacy of products that claim to kill mildew is dependent upon concentration and length of time the active ingredient is in contact with the organism. Methods for testing such products are included in § 93-30 Items 5 and 6. The test method should be modified for surfaces other than hard, nonporous surfaces.

(c) Mildewcides and mildewstats should also be tested to determine whether or not bleaching, staining, spotting or other undesirable effects occur on the surfaces, articles, and materials to be protected.

§ 93-16 Products for control of organisms producing mycotoxins (Reserved)

(a) See also § 93-7 Products for use as grain preservatives.

(b) The registrant is advised to consult with the Agency on their test protocol before initiating product performance testing.

§ 93-30 Acceptable methods.

I. Surfaces (Mold and Mildew)

Item 1 - Fabric Mildew Fungistatic Test Method

1. Scope.

1.1 Products intended for use to control, prevent, or inhibit the growth of fungi which cause mildew on various articles or surfaces should be tested to demonstrate fungistatic effectiveness. This method is designed to determine effectiveness of products intended to control mildew and nonpathogenic fungal growth on indoor articles or surfaces composed of fabric. It also indicates the duration of protection afforded, thereby providing a basis for recommending when applications are to be repeated. This method is not applicable for evaluating laundry additive products unless the appropriate modifications are made to simulate actual use conditions.

2. Summary of Method.

2.1 Treated, dried strips of cotton fabric are sprayed with a mixed spore suspension of mildew causing organisms and incubated at high humidity. Mildew growth on treated and untreated cotton strips is rated at weekly intervals for up to four weeks.

3. Apparatus.

3.1 Glassware: Flasks with cotton plugs suitable for preparation of agar, diluent, and conidial suspensions. French square jars (500 ml) or equivalent screw cap containers. Caps modified by center drilling and inserting an appropriate size stainless steel or brass bolt to which a hook (formed from a 6-7 cm length of #22 nickel-chromium wire or other non-corrosive wire) is attached. Hook position adjusted so that the bottom ends of attached cotton fabric samples when in jars are about 13 mm above water. (See 7.5.)

3.2 Tissue grinder (Homogenizer) No. 4288B, Arthur H. Thomas Co., Phila., Pa.

3.3 Atomizer, DeVilbiss #152 (or equivalent) operated at 69 kPa (10 psi).

3.4 Counting chamber: Suitable for determining spore concentrations.

4. Test Specimens.

4.1 Cut 25 by 75 mm strips from 136 to 203 g/m^2 (4 to 6 oz/yd^2) cotton muslin 1/. Fabric that will hang without curling excessively is preferable. NOTE: The above test fabric is suitable for testing products intended for use on general household fabric. Products intended solely for use on heavier fabrics must be tested on cotton fabric weighing approximately the same as that of the lowest fabric weight to be treated under actual use conditions.

5. Test Fungi.

5.1 Aspergillus niger (ATCC 6275) 3/ and Penicillium variabile (NRRL-3765 or ATCC 32333) 4/. Maintain stock cultures of each on neopeptone agar (10g neopeptone, 20g dextrose, 20g agar, and 1 liter distilled water) or Emmons Agar. Incubate new stock cultures 7 to 10 days at 25°C, then store at 2 to 10°C.

6. Selection of Treatments.

6.1 Test Fungistat: Dosages of the test fungistat evaluated should range from ineffective to effective levels so that the minimum effective dosage of test material can be determined. NOTE: Where both wipe-on and pump spray (i.e., non-pressurized containers) application methods are intended for the proposed formulation, only the wipe-on applications method need to be tested.

6.2 Untreated Control: Ten untreated fabric strips are required to establish the test validity and ascertain the degree of control obtained with the test fungistat.

6.3 Standard Fungistat: A fungistat registered for use on fabric may be included in the test as a comparative treatment. The product selected must be used in accordance with label directions and should involve a method of application comparable to that of the test fungistat.

7. Procedures. NOTE: Aseptic procedures must be followed throughout the course of the test.

7.1 Preparation of Conidial Suspensions: Conidial suspensions of fungal organism are prepared by washing spores from the surface of 7 to 10 day agar cultures with sterile 0.85% saline solution containing a surfactant such as 0.05% isooctylphenoxypolyethoxyethanol. 5/ Spore chains may be broken up by transferring suspension to a heat sterilized tissue grinder and reciprocating the piston several times. Hyphal fragments should be removed by filtering the suspension through a thin layer of sterile cotton or other suitable material. Conidial suspension may be stored at 2 to 10°C for up to four weeks. Inoculum for test should be adjusted to contain five million conidia per ml on the day of use by appropriate dilution of stock suspension with saline solution. 6/

7.2 Preparation of test specimen: Invalid tests due to failure of mildew growth on the untreated test fabric can be reduced or eliminated by applying a nutrient solution to the fabric. To insure luxuriant growth on fabric strips saturate fabric with a sterilized glycerol nutrient solution of the following composition: 98.7% distilled water, 1.0% glycerol, 0.1% potassium phosphate, monobasic, 0.1%

ammonium nitrate, 0.025% magnesium sulfate (heptahydrate), and 0.05% yeast extract. 7/ Adjust solution to pH 5.3. Sufficient nutrient solution should be prepared to saturate all the fabric to be used with a single test. Soak the fabric in nutrient for three minutes or until saturated. Squeeze excess liquid with the fingers and allow fabric strips to dry before proceeding with application of test products or standard fungistat.

7.3 Treatment: Treat both sides of ten fabric strips for each formulation, dosage rate, and method of application being evaluated. For spray applications, the type of sprayer and distance from nozzle to cloth surface, as well as the degree of wetness, must be controlled and specified. If a standard fungistat is used, ten fabric strips should be treated in accordance with label directions for use. Immediately after treatment, fabric strips should be placed in a vertical or near vertical position to permit excess liquid to drain. Include ten untreated strips as controls. All samples are allowed to dry before proceeding.

7.4 Inoculation: Place equal volumes of well-agitated conidial suspensions of *A. niger* and *P. variabile* in a DeVilbiss atomizer (or equivalent), maintain agitation and lightly spray both sides of each fabric strip.

7.5 Incubation: The fabric samples are then suspended in individual 500 ml jars containing approximately 90 ml water, and incubated at approximately 28°C. The caps are tightened, then backed off 1/8 turn to allow for some ventilation.

8. Determination of Results.

8.1 Evaluation: Observations are made and recorded weekly for four weeks, or until treatments fail and abundant growth occurs on all treated strips. The presence or absence of observable mold on the fabric strips is the criterion for determining the effectiveness of the test product. Where no growth is visually evident at the end of the test period, examination at approximately 15X magnification must be conducted to confirm the absence or establish the presence of subvisual growth. The untreated control strips must have a minimum of 50% of their surface area covered with fungal growth after 7 days to consider the test valid.

8.2 Interpretation: A product dosage is considered acceptable when all ten treated replicates are free of fungal growth. The results of this test must be correlated to intended label claims. The directions for use must specify retreatment every 7, 14, or 21 days, as necessary depending on the length of time that all of the test strips remain free of mildew growth. Labeling of products which do not permit growth after four weeks incubation must specify a retreatment schedule, such as "repeat as necessary when new growth appears", and should indicate that treatments should be effective for at least 28 days.

9. Data Reporting.

9.1 Test reports must include all pertinent details of the test conditions and variables. Such information shall include at least the following types of information:

9.1.1 Complete description of formulations tested (type of formulation, name and percentage of active ingredients, and EPA Registration Number of any standard fungistat used).

9.1.2 Dosage rates (specify whether rate is in terms of product or active ingredients, and whether on a weight and/or volume basis).

9.1.3 Complete description of all appropriate application procedures and materials including details such as the type of sprayer (pump vs. pressurized spray), spray application distance and duration, applicator material used for wipe-on applications (damp cloth), degree of wetness obtained on surfaces (dampen, thoroughly wet, etc.), and time interval between application and rinsing.

9.1.4 Density (weight/unit area) of test fabric.

9.1.5 Test validity data--number of untreated controls wiith 50% or more of the surface area covered with fungal growth after 7 days.

9.1.6 Effectiveness data--number of replicates with fungal growth at each observation date for each treatment being evaluated (including untreated controls). To demonstrate differences among treatments, it may be necessary to use additional criteria, such as the percentage of surface area covered with fungal growth or the density of fungal growth.

9.1.7 Adverse effects data--describe the nature and extent of any adverse effects noted on the fabric as a result of treatment.

9.1.8 Modifications--describe the nature of any changes made in the test method and provide the rationale for each change.

Footnotes

1/ Cotton muslin and heavier types of cotton fabric are available from Test-fabrics Inc., 200 Blackford Avenue, P.O. Box 53, Middlesex, N.J. 08846.

2/ DeVilbiss Atomizer available from DeVilbiss Co., Toledo, Ohio 43682.

3/ Cultures of *A. niger* (ATCC 6275) are available from: American Type Culture Collection, 12301 Parklawn Dr., Rockville, Md. 20852.

4/ Cultures of P. variabile (NRRL 3765 or ATCC 32333) are available from: ARS Culture Collection Investigations Fermentation Laboratory, USDA, 1815 North University St., Peoria, Ill. 61604, or American Type Culture Collection, 12301 Parklawn Dr., Rockville, Md. 20852.

5/ Triton X-100, Rohm & Haas Co., Phila., Pa. 19104, or other suitable wetting agent such as dioctyl sodium sulfosuccinate, or Aerosol OT solid A-349, Fisher Scientific Co.

6/ For detailed instructions: Tuite, John, Plant Pathological Methods, Fungi and Bacteria, Burgess Publishing Co., Minneapolis, Minn., 1969, pp. 183-184.

7/ The glycerol as a humectant provides a more equal distribution of moisture to all areas of the substrate. The glycerol, yeast extract and mineral salts also provide the nutrients necessary for fungal growth.

Item 2 - Hard Surface Mildew Fungistatic Test Method

1. Scope.

1.1 Products which are intended for use to control, prevent, or inhibit the growth of fungi which cause mildew on various articles or surfaces should be tested to demonstrate fungistatic effectiveness. This method is designed to determine effectiveness of products intended to control mildew and non-pathogenic fungal growth on indoor hard nonporous surfaces, such as painted walls, ceilings, floors, metal, glass, tile, porcelain, and plastic. This method is not satisfactory to demonstrate whether or not the product will produce adverse effects on plastic, painted, or metal articles and surfaces.

2. Summary of Method.

2.1 The test is conducted by treating the glazed side of square sections of tile. After drying, the glazed side of tiles are sprayed with an inoculum-nutrient solution, redried, and incubated individually in petri dishes. Mildew growth on treated and untreated tiles is visually rated after 7 days of incubation.

3. Apparatus.

3.1 Glassware: Flasks with cotton plugs suitable for preparing agar, solutions, and spore suspensions. Petri plates as containers for drying and incubation of tiles.

3.2 Tissue grinder (Homogenizer): No. 4288B, Arthur H. Thomas Co.

3.3 Atomizer: DeVilbiss #152 (or equivalent) operated at 69 kPa (10 psi).

3.4 Counting chamber: Suitable for determining spore concentration.

4. Test Specimens.

4.1 Ceramic tiles: 25 mm square tiles with glazed surface, sterilized for 2 hours in hot air oven at 180°C.

5. Test Fungus and Materials.

5.1 Aspergillus niger (ATCC 6275). 1/ Maintain stock culture on neopeptone agar (10 neopeptone, 20g dextrose, 20g agar, and 1 liter distilled water) or Emmons agar. Incubate new stock cultures 7 to 10 days at 25°C, then store at 2 to 10°C.

5.22. Water agar: Two percent water agar (20g agar per liter of distilled water).

5.3 Sterile Czapek solution: Distilled water 1000 ml; sodium nitrate 3g; potassium phosphate dibasic 1g; potassium chloride 0.5g; magnesium sulfate 0.5g; ferrous sulfate 0.01g; sucrose 30g.

5.4 Sterile saline solution: 0.85% sodium chloride and 0.05% isooctylphenoxypolyethoxyethanol 2/ in distilled water.

6. Selection of Treatments.

6.1 Test Fungistat: Dosages of the test fungistat should range from ineffective to effective levels so that the minimum effective dosage of the test material can be determined. NOTE: If both wipe-on and pump spray (i.e., non-pressurized containers) methods of applications are intended for the proposed formulation, only the wipe-on application method needs to be tested.

6.2 Untreated Control: Ten untreated glazed tiles are included for purposes of determining the validity of the test and the degree of control obtained with the test fungistat.

6.3 Standard Fungistat: A fungistat registered for use on hard, non-porous surfaces may be included in the test as a comparative treatment. The product selected must be used in accordance with label directions and should involve a method of application comparable to that of the test fungistat.

7. Procedures. NOTE: Aseptic procedure must be followed throughout the test.

7.1 Preparation of Conidial Suspension: Wash spores from the surface of 7 to 10 day cultures of test fungus with sterile saline solution. Pour resulting spore suspension into a sterilized tissue grinder, reciprocate piston several times to break up spore chains. Filter suspension through a thin layer of sterile cotton or other suitable material to remove spore chains and hyphal elements. Conidial suspensions may be stored at 2 to 10° for up to four weeks. Standardize conidial suspensions to contain five million conida per ml. Determine spore concentrations using a counting chamber 3/ and adjust to proper concentration with saline solution. Place 1 ml of the standard conidial suspension in 20 ml sterile Czapek's liquid medium and agitate.

7.2 Treatment: Treat the glazed side of ten tiles for each formulation, dosage, and method of application being evaluated. For spray applications the type of sprayer and distance from nozzle to tile surface, as well as the degree of surface wetness, must be controlled and specified. If a standard fungistat is used, ten tiles should be treated in accordance with the label directions for use. Immediately after treatment, tiles should be placed in a vertical or near vertical position to permit excess liquid to drain. Place five tiles in each of two sterile petri dishes and allow to dry at 37°C with lids ajar. Include ten untreated tiles, as controls.

7.3 Inoculation: Place well agitated A. niger conidial-nutrient suspension in a #152 DeVilbiss atomizer (or equivalent), maintain agitation and lightly spray the glazed surface of each tile. Tiles contained in petri dishes (with lids ajar) are then returned to 37°C oven and dried.

7.4 Incubation: Each tile, sprayed side up, is then placed in an individual petri dish containing hardened sterile water agar. Plates are incubated at 25°C and a minimum of 95% relative humidity (a wet-type incubator has been found suitable for this purpose).

8. Determination of Results.

8.1 Evaluation: Observations are made and recorded after 7 days of incubation. The presence or absence of observable fungal growth on tiles is the criterion for determining the effectiveness of the test product. When no visual growth is evident at the end of the test period, examination at approximately 15X magnification must be conducted to determine the presence or absence of growth. Untreated control tiles must be at least 50% covered with fungal growth after 7 days in order to consider the test valid.

8.2 Interpretation: A product dosage is considered acceptable when all ten treated replicates are free of fungal growth. The results of this test must be correlated with the intended label claims. Products which do not permit growth after 7 days must specify a retreatment schedule, such as, "repeat as necessary when new growth appears". Product labeling must state some of the appropriate hard, nonporous surfaces (such as those listed in scope of method) on which the product is intended to be used.

9. Data Reporting.

9.1 Test reports must include all pertinent details of the test conditions and variables. Such information shall include at least the following types of information:

9.1.1 Complete description of formulation(s) tested (type of formulation, name and percentage of active ingredient(s), and EPA Registration Number of any standard fungistat used).

9.1.2 Dosage rates (specify whether rate is in terms of product or active ingredient, and whether on a weight and/or volume basis).

9.1.3 Complete description of all appropriate application procedures and materials including details such as the type of sprayer (pump vs. pressurized sprayer), spray application distance and duration, applicator material used for wipe-on applications (damp cloth), degree of wetness to be obtained on surfaces (dampen, thoroughly wet, etc.), and time interval between application and rinsing.

9.1.4 Test validity data--the number of untreated controls with 50% or more of the surface area covered with fungal growth after 7 days.

9.1.5 Effectiveness data--the number of replicates with fungal growth on observation date for each treatment being evaluated (including untreated controls). To demonstrate differences among treatments it may be necessary to use additional criteria, such as the percentage of surface area covered with fungal growth or the density of fungal growth.

9.1.6 Adverse effects data--describe the nature and extent of any adverse effects noted on the glazed tiles as a result of treatment.

9.1.7 Modifications--describe the nature of any changes made in the test method and provide the rationale for each change.

Footnotes

1/ Cultures of A. niger (ATCC 6275) are available from: American Type Culture Collection, 12301 Parklawn Dr., Rockville, Md. 20852.

2/ Triton X-100, Rohm & Haas Co., Phila., Pa. 19104, or other suitable wetting agent.

3/ For detailed instructions: Tuite, John. 1969. Plant Pathological Methods, Fungi and Bacteria. Burgess Publishing Co., Minneapolis, Minn. Pp. 183-184.

Item 3 - Leather Mildew Fungistatic Test Method

1. Scope.

1.1 Products intended for use to control, prevent or inhibit the growth of fungi which cause mildew on various articles or surfaces should be tested to demonstrate fungistatic effectiveness. This method is designed to determine effectiveness of products intended to control mildew and non-pathogenic fungal growth on indoor articles or surfaces of leather, such as book covers, luggage, shoes, and sporting goods. It also indicates duration of protection afforded, thereby providing a basis for recommending when to repeat applications.

2. Summary of Method.

2.1 This method simulates use conditions by utilizing sections of vegetable tanned cowhide which, after treatment with the product, are artificially inoculated with a spore suspension of mildew-causing organisms and incubated at high humidity. Mildew growth on treated and untreated leather surfaces is rated during the four week test.

3. Apparatus.

3.1 Glassware: Flasks with cotton plugs suitable for preparation of agar, diluent, and conidial suspensions. French square jars (500 ml) or equivalent screw cap containers. Screw caps adapted to allow suspension of leather sections 30 to 40 mm below the jar caps. Caps modified by center drilling cap and inserting appropriate size stainless steel or brass bolt to which a hook (formed from a 6-7 cm length of #22 nickel-chromium wire or other non-corrosive wire) is attached.

3.2 Tissue grinder (homogenizer): No. 4288B, Arthur H. Thomas, Co.

3.3 Atomizer: DeVilbiss #152 (or equivalent) operated at 69 kPa (10 psi).

3.4 Counting chamber: Suitable for determining spore concentration.

4. Test Specimens.

4.1 Leather: Squares (25mm) of vegetable tanned cowhide (1.0 to 1.5 mm thick) 1/ with a hole punched in the corner of each square to permit it to be suspended from hook on modified French jar lid.

5. Test Fungi.

5.1 Aspergillus niger (ATCC 6275) 2/ and Penicillium variabile (NRRL-3765 or ATCC 32333). 3/ Maintain stock cultures of each organism on neopeptone agar (10g neopeptone, 20g dextrose, 20g agar, and 1 liter distilled water) or Emmons agar. Incubate new stock cultures 7 to 10 days at 25°C, then store at 2 to 10°C.

6. Selection of Treatments:

6.1 Test Fungistat: Dosages of the test fungistat evaluated should range from ineffective to effective levels so that the minimum effective dosage of test material can be determined. NOTE: Where both wipe-on and pump spray (i.e., non-pressurized containers) application methods are intended for the proposed formulation, only the wipe-on application method need to be tested.

6.2 Untreated Control: Ten untreated leather squares are required to establish the test validity and to ascertain the degree of control obtained with the test fungistat.

6.3 Standard Fungistat: A fungistat registered for use on leather may be included in the test as a comparative treatment. The product selected must be used in accordance with label directions and should involve a method of application comparable to that of the test fungistat.

7. Procedures.

NOTE: Aseptic procedures must be followed throughout the course of the test.

7.1 Preparation of conidial suspensions: Conidial suspensions of each fungal organism are prepared by washing spores from the surface of 7- to 10-day neopeptone agar cultures with sterile 0.85% saline solution containing a surfactant such as 0.05% isooctylphenoxypolyethoxyethanol. 4/ Spore chains should be broken up by transferring the suspension to the heat-sterilized tissue grinder and reciprocating the piston several times. Hyphal fragments should be removed by filtering the suspension through a thin layer of sterile cotton or other suitable material. Conidial suspension may be stored at 2 to 10°C for up to four weeks. Standardize test conidial suspensions to contain five million conidia per ml (determine spore concentration with a counting chamber 5/) by adding sterile saline solution.

7.2 Treatment of Leather Squares: Treat both sides of ten leather test squares for each formulation, dosage and method of application being evaluated. Wipe on/or spray application must simulate intended method of use. For spray applications, the type of sprayer and distance from nozzle to leather surface, as well as the degree of surface wetness, must be controlled and specified. Dip application of product to leather test squares is not an acceptable substitute for wipe-on or spray methods of application. If a standard fungistat is used, the leather squares should be treated in accordance with label directions for use. Immediately after treatment leather squares should be placed in a vertical or near vertical position to permit excess liquid to drain. All treated leather should be allowed to dry before the mildew spore suspension is applied.

7.3 Inoculation: Place equal volumes of well agitated A. niger and P. variabile conidial suspensions in a #152 DeVilbiss atomizer (or equivalent), maintain agitation and lightly spray both sides of each leather square with the mixture.

7.4 Incubation: Suspend leather squares in individual modified 500 ml French square jars containing approximately 90 ml distilled water and incubate at approximately 28°C. Tighten the caps, then back off 1/8 turn to allow for some ventilation.

8. Determination of Results.

8.1 Evaluation: Observations are recorded weekly for four weeks or until treatments fail and abundant growth occurs on all treated squares. The presence or absence of observable mold on leather squares is the criterion for determining the effectiveness of the test product. Where no visual growth is evident at the end of the test period, examination at approximately 15X magnification is required to confirm the absence or establish the presence of subvisual fungal growth. The untreated control squares must have a minimum of 50% of their surface area covered with fungal growth after 7 days for the test to be valid.

8.2 Interpretation: A product dosage is considered acceptable when all 10 treated replicates are free of fungal growth. The results of this test must be correlated to the intended label claims. The directions for use must be correlated to the intended label claims. The directions for use must specify retreatment every 7, 14, and 21 days, depending on the length of time all treated test squares remained free of mildew growth. Label directions for products which remain effective for the duration of the four week test must specify a retreatment schedule, such as "repeat as necessary when new growth appears" and should indicate that treatments be effective for at least 28 days.

9. Data Reporting.

9.1 Test reports must include all pertinent details of the test conditions and variables. Such information shall include at least the following types of information:

9.1.1 Complete description of formulation(s) tested (type of formulation, name and percentage of active ingredient(s), and EPA Registration Number of any standard fungistat used).

9.1.2 Dosage rates (specify whether rate is in terms of product or active ingredient, and whether on a weight and/or volume basis).

9.1.3 Complete description of all appropriate application procedures and materials including details such as the type of sprayer (pump vs. pressurized spray), spray application distance and duration, applicator material used for wipe-on applications (damp cloth), degree of wetness to be obtained on surfaces (dampen, thoroughly wet, etc.), and time interval between application and rinsing.

9.1.4 Test validity--the number of replicates with 50% or more of the surface area covered with fungal growth after 7 days.

9.1.5 Effectiveness--the number of replicates with fungal growth at each observation date for each treatment being evaluated (including untreated controls). To demonstrate the differences among treatments it may be necessary to use additional criteria, such as the percentage of surface area covered with fungal growth or the density of fungal growth.

9.1.6 Adverse effects data--describe the nature and extent of any adverse effects noted on leather as a result of treatment.

9.1.7 Modification--describe the nature of any changes made in the test method and provide the rationale for each change.

Footnotes

1/ Vegetable tanned leather is available from Eberle Tanning Company, 360 Church Street, Westfield, Pennsylvania 16950.

2/ Cultures of A. niger (ATCC 6275) may be obtained from American Type Culture Collection, 12301 Parklawn Dr., Rockville, Md. 20852.

3/ Cultures of P. variabile (NRRL 3765)(ATCC 32333) are available from: ARS Culture Collection Investigations Fermentation Laboratory, USDA, 1815 North University St., Peoria, Ill. 61604, or American Type Culture Collection, 12301 Parklawn Dr., Rockville, Md. 20852.

4/ Triton-X-100, Rohm & Haas Co., Phila., Pa. 19104, or other suitable wetting agent such as dioctyl sodium sulfosuccinate, as Aerosol OT solid A-349, Fisher Scientific Co.

5/ For detailed instructions see: Tuite, John, Plant Pathological Methods, Fungi and Bacteria, Burgess Publishing Co., Minneapolis, Minn., 1969, pp. 183-184.

Item 4 - Wood Block Mildew Fungistatic Test Method

1. Scope.

1.1 Products intended for use to control, prevent or inhibit the growth of fungi which cause mildew on various articles or on surfaces should be tested to demonstrate fungistatic effectiveness. This method is designed to determine effectiveness of products intended to control mildew and non-pathogenic fungal growth on indoor articles or surfaces composed of wood which have not been painted or coated. It also indicates duration of protection afforded, thereby providing a basis for recommending when to repeat applications. This test is not satisfactory to support claims to control rot and decay of wood, or mold and stain on fresh-cut lumber.

2. Summary of Method.

2.1 This test utilizes wooden blocks which are treated with the test products and then sprayed with a mixed spore suspension of test fungi. During incubation the mildew growth on treated and untreated wood blocks are visually rated at weekly intervals for four weeks.

3. Apparatus.

3.1 Glassware: Flasks with cotton plugs suitable for preparing agar, solutions, and spore suspensions. French square jars (500 ml) or equivalent screw cap containers, with modified caps (10 jars per treatment evaluated). Jar caps modified by center drilling the caps and inserting stainless steel or brass bolts to which hooks (formed from a 63 mm piece of #22 nickel chromium wire) are attached for suspending the test samples. Length of the wire from cap to the bottom of the hook approximately 5 cm.

3.2 Tissue grinder (Homogenizer) No. 4288B Arthur H. Thomas Co.

3.3 Atomizer, DeVilbiss #152 (or equivalent operated at 69 kPa (10 psi).

3.4 Counting chamber: Suitable for determining spore concentration.

4. Test Specimens.

4.1 Pine sapwood blocks 25 by 25 x 15 mm. The wood should be free of excessive resins, knots, growth rings, and other defects, and contain no heartwood. Block should be smooth-surfaced on all six sides, and kiln dried after sawing to avoid infestation by wood rotting fungi. Each block is corner drilled to allow hanging as described in 3.1; other means of hanging can be used.

5. Fungi.

5.1 Aspergillus niger (ATCC 6275) 1/ and Penicillium variabile (NRRL-3765, or ATCC 32333) 2/. Maintain separate stock cultures on neopeptone agar (10g neopeptone, 20g dextrose, 20g agar, and 1 liter distilled water) or Emmons agar. Incubate new stock cultures seven to ten days at 25°C, then store at 2 to 10°C.

6. Selection of Treatments.

6.1 Test Fungistat: A fungistat registered for use on unpainted wood surfaces may be included in the test as a comparative treatment. The product selected must be used in accordance with label directions and should involve a method of application comparable to that of the test fungistat.

7. Procedures. NOTE: Aseptic procedures must be followed throughout the test.

7.1 Preparation of Conidial Suspensions: Separate conidial suspensions of each fungal organism are prepared. Prepare a sterile saline solution which contains 0.85% sodium chloride and 0.5% isooctylphenoxypolyethoxyethanol 3/ in distilled water. Wash spores from the surface of 7- to 10-day neopeptone agar cultures with the sterile saline solution. Pour the spore suspension into a heat sterilized tissue grinder, and reciprocate the piston several times to break up spore chains. Filter suspension through a thin layer of sterile cotton or other suitable material to remove spore chains and hyphal elements. Conidial suspensions may be stored at 2 to 10°C for up to four weeks. Standardize test conidial suspensions to contain five million conidia per ml (determine spore concentration with a counting chamber) 4/ by adding sterile saline solution.

7.2 Treatment: Treat all sides of ten wood blocks for each formulation, dosage rate, and method of application being evaluated. For spray applications, all sides should be sprayed at a specified distance to obtain the desired degree of wetness. If a standard fungistat is used, wood blocks should be treated in accordance with the label directions for use. Include ten untreated blocks as controls. All samples are allowed to dry before proceeding.

7.3 Inoculation: Place equal volumes of well agitated _A. niger_ and _P. variabile_ conidial suspensions in a #152 DeVilbiss atomizer (or equivalent), maintain agitation, and lightly spray all surfaces of the test blocks.

7.4 Incubation: The blocks are then suspended in individual jars above approximately 90 ml of water, and incubated at approximately 28°C. The caps are tightened, then backed off 1/8 turn to allow for some ventilation.

8.1 Evaluation: Observations are recorded weekly for four weeks or until abundant growth occurs on treated blocks. The presence or absence of observable mold on wood blocks is the criterion for determining the effectiveness of the test product. Where no visual growth is evident at the end of the test period, examination at approximately 15x magnification must be conducted to determine the presence or absence of subvisual growth. The untreated control blocks must have a minimum of 50% of their surface area covered with fungal growth after 7 days for the test to be considered valid.

8.2 Interpretation: A product dosage is considered acceptable when all ten treated replicates are free of fungal growth. The results of this test must be correlated to the intended label claims. The directions for use must specify retreatment every 7, 14, or 21 days, as necessary, depending on the length of time that all of the test blocks remain free of mildew growth. Products which do not permit growth after four weeks incubation must specify a retreatment schedule, such as "repeat as necessary when new growth appears" and should indicate that treatments should be effective for at least 28 days.

9. Data Reporting.

9.1 Test reports must include all pertinent details of the test conditions and variables. Such information shall include at least the following types of information:

9.1.1 Complete description of formulation(s) tested (type of formulation, name and percentage of active ingredient(s), and EPA Registration Number of any standard fungistat used).

9.1.2 Dosage rates (specify whether in terms of product or active ingredient and whether on a weight and/or volume basis).

9.1.3 Complete description of all appropriate application procedures and materials including details such as the type of sprayer (pump vs. pressurized spray), spray application distance and duration, applicator materials used for wipe-on applications (damp cloth), degree of wetness to be obtained on surfaces (dampen, thoroughly wet, etc.), and time interval between application and rinsing.

9.1.4 Test validity data--the number of untreated controls with 50% or more of the surface area covered with fungal growth after 7 days.

9.1.5 Effectiveness data--the number of replicates with fungal growth at each observation date for each treatment being evaluated (including untreated controls). Differences among treatments may be demonstrated by use of additional criteria, such as the percentage of surface area covered with fungal growth or the density of fungal growth.

9.1.6 Adverse effects data--describe the nature and extent of any adverse effects noted on wood blocks as a result of treatment.

9.1.7 Modifications--describe the nature of any changes made in the test method and provide the rationale for each change.

Footnotes

1/ Cultures of *A. niger* (ATCC 6275) are available from: American Type Culture Collection, 12301 Parklawn Dr., Rockville, Md. 20852.

2/ Cultures of *P. variabile* (NRRL-3765, ATCC 32333) are available from: ARS Culture Collection Investigations Fermentation Laboratory, USDA, Northern Utilization Research and Development Division, 1815 North University Street, Peoria, Ill.; or American Type Culture Collection, 12301 Parklawn Dr., Rockville, Md. 20852.

3/ Triton X-100, Rohm & Haas Co. Phila., Pa. 19104, or other suitable wetting agent such as dioctyl sodium sulfosuccinate (Aerosol OT solid A-349, Fisher Scientific Co.).

4/ For detailed instructions: Tuite, John, *Plant Pathological Methods, Fungi and Bacteria*, Burgess Publishing Co., Minneapolis, Minn., 1969, pp. 183-184.

Item 5 - Glass Slide Mildew Fungicidal Test Method

1. Scope.

1.1 Products intended for use to kill fungi which cause mold and mildew growth should be tested to demonstrate "cidal" effectiveness. This method is designed to determine the effectiveness of products intended to *kill* mildew organisms on hard, nonporous surfaces. Residual effectiveness if intended or claimed must be demonstrated using other tests (i.e., Fabric Mildew Fungistatic Test Method, Item 1; Hard Surface Mildew Fungistatic Test Method, Item 2; Leather Mildew Fungistatic Test Method, Item 3; Wood Block Mildew Fungistatic Test Method, Item 4) depending on the nature of the surfaces or articles on which the product is intended to be used. This method is especially applicable for testing products applied as sprays or formulated as pressurized sprays.

2. Summary of Method.

2.1 This method is conducted using specially prepared sterile square glass slides which are seeded with a standardized spore suspension of the test organism. The slides are sprayed individually with the test fungicide and then placed into large test tubes containing culture medium plus a fungicide neutralizer for incubation and subsequent evaluation for the presence or absence of fungal growth.

3. Apparatus.

3.1 Glassware: Lipless 32 x 200 mm test tubes with plugs. Petri dishes 15 x 100 mm. Flasks with plugs. All glassware sterilized two hours in hot air oven at 180°C.

3.2 Transfer loop (or equivalent device) which will deliver approximately 0.01 ml of spore suspension.

3.3 Racks and baskets: Suitable for holding test tubes.

3.4 Microscope slides: Noncorrosive (25 x 25 mm) slides placed individually in a petri dish matted with two pieces of 90 mm diameter filter paper (Whatman No. 1 or equivalent) and placed in a hot air oven for sterilization.

3.5 Tissue grinder (Homogenizer) No. 4288B Arthur H. Thomas Co.

3.6 Counting chamber suitable for determining spore concentration.

4. Reagents and Materials.

4.1 Distilled water or water of equal purity.

4.2 Neopeptone--neutralizer broth: prepare by dissolving 0.7g lecithin and 5.0g sorbitan monooleate 1/ in 400 ml of hot water and boiling until dissolved. Add 1.0g sodium thiosulfate, 10g neopeptone and 20g dextrose, and sufficient water to make one liter of culture medium. The pH of the medium will be approximately 7.2 after autoclaving.

4.4 Saline solution as 0.85% sodium chloride and 0.05% isooctylphenoxypolyethoxyethanol 2/ in distilled water.

5. Test Fungus.

5.1 Aspergillus niger (ATCC 6275) 3/. Maintain stock cultures on neopeptone agar (10g neopeptone, 20g dextrose, 20g agar and 1 liter distilled water). Incubate stock culture for 7 to 10 days at 25°C, then store at 2 to 10°C.

6. Selection of Treatments.

6.1 Test Fungicide: A sufficient number of dosages of the test fungicide should be evaluated in order to determine the minimum effective dosage.

6.2 Untreated Control: Two untreated slides are included for determining the validity of the test.

6.3 Standard Fungicide: A fungicide registered for use on similar surfaces may be included in the test as a comparative treatment. The product selected must be used in accordance with label directions and should involve a method of application comparable to that of the test fungicide.

7. Procedures. NOTE: Aseptic procedures must be followed throughout the course of the test.

7.1 Preparation of Conidial Suspension: Conidial suspensions of the fungus are prepared by washing the spores from the surface of 7- to 10-day old neopeptone agar cultures with sterile saline solution. The spore suspension is poured into a heat sterilized tissue grinder and the piston reciprocated several times to break up the spore chains. Filter suspension through a thin layer of sterile cotton or other suitable material to remove spore chains and hyphal elements. Conidial suspensions may be stored at 2 to 10°C for up to four weeks. Standardize test conidial suspensions to contain five million conidia per ml by adding sterile diluent. Determine spore concentration with a counting chamber 4.

7.2 Inoculation: Agitate spore suspension to disperse spores evenly throughout, transfer approximately 0.01 ml of the spore suspension by means of a transfer loop onto each 25 mm square sterile test slide (contained in a petri dish) and spread evenly over the upper surface. Cover the dish immediately and repeat the procedure until twelve slides have been prepared (use two slides as controls). Allow all slides to dry for 40 minutes at 37°C or let stand several hours at room temperature.

7.3 Treatment: Spray ten inoculated slides with the test product concentration at a specified distance to obtain the desired degree of wetness. Immediately after treatment, drain excess liquid from slides and maintain in a petri dish for an exposure of one minute. NOTE: Products which are capable of keeping surfaces totally wet for longer than one minute under actual use conditions, should be tested under a longer exposure time. To determine the duration of such an increased exposure time the following test procedure should be employed. Tests must utilize a hard nonporous surface (e.g., glass, metal, or porcelain) of at least one square foot in area which are treated in accordance with the proposed label directions for use. The test surface(s) must be positioned vertically, unless the product is intended solely for use on horizontal surfaces (e.g., floors) in which case horizontal positions must be used. The test must be conducted at a temperature of 20 to 25°C and a relative humidity of 50% or less. The length of time (in seconds) from application to when any portion of the treated surface, begins to appear dry should be recorded. The average length of this drying time for a minimum of three replicates shall serve as the basis for determining the increased treatment exposure time. Products which keep surfaces totally wet for longer than 10 minutes should utilize a 10 minute exposure time.

7.4 Incubation: Transfer each slide by means of flamed forceps to separate 32 x 200 mm test tubes containing 20 ml of neopeptone neutralizer broth. Shake culture medium thoroughly. Transfer two unsprayed slides, as viability controls, to individual culture tubes in the same manner. Incubate all tubes at 25°C for at least three days.

8. Determination of Results.

8.1 Evaluation: The presence or absence of fungal growth, after 3 days, is the criterion for determining "cidal" effectiveness of the test product. For a valid test, fungal growth must be present in both viability control replicates.

8.2 Interpretation: A product dosage is considered acceptable when all ten treated replicates are free of fungal growth. The results of this test must be correlated with the intended label claims. Products which pass this test may be labeled as fungicides or mildewcides which kill mold and mildew organisms. If the product is not tested for residual effectiveness, the labeling must state "non-residual" or "kills on contact."

9. Data Reporting.

9.1 Test reports must include all pertinent details of the test conditions and variables. Such information shall include:

9.1.1 Complete description of formulation(s) tested (type of formulation, name and percentage of active ingredient(s), and EPA Registration Number of any standard fungicide used).

9.1.2 Dosage rates (specify whether in terms of product or active ingredient, and whether on a weight and/or volume basis).

9.1.3 Complete description of all appropriate application procedures and materials including details such as the type of sprayer (pump vs. pressurized spray), spray application distance and duration, degree of wetness to be obtained on surfaces (dampen, thoroughly wet, etc.), and time interval between application and rinsing.

9.1.4 Effectiveness data--the number of replicates with fungal growth for each treatment being evaluated (including untreated controls).

9.1.5 Modifications--describe the nature of any changes made in the test method and provide the rationale for each change. For example: the duration of any exposure time which is longer than 1 minute exposure specified in section 7.3, plus the results of the drying time test used to support the change in exposure time should be reported.

Footnotes

1/ Tween-80, ICI United States, Agricultural Division, Wilmington, Del. 19898.

2/ Triton X-100, Rohm & Haas Co., Phila., Pa. 19104, or other suitable agent such as dioctyl sodium sulfosuccinate (Aerosol OT solid A-349, Fisher Scientific Co.).

3/ Cultures of A. niger (ATCC 6275) may be obtained from American Type Culture Collection, 12301 Parklawn Dr., Rockville, Md. 20852.

4/ For detailed instructions: Tuite, John. 1969. Plant Pathological Methods, Fungi and Bacteria. Burgess Publishing Co., Minneapolis, Minn. Pp. 183-184.

Item 6 - Use-Dilution Mildew Fungicidal Test Method

1. Scope.

1.1 Products intended for use to kill fungi which cause mold and mildew growth should be tested to demonstrate "cidal" effectiveness. This method is designed to determine the effectiveness of products intended to kill mildew organisms on hard non-porous surfaces. Residual effectiveness, if intended, must be demonstrated using other tests (i.e., Fabric Mildew Fungistatic Test Method, Item 1; Hard Surface Mildew Fungistatic Test Method, Item 2; Leather Mildew Fungistatic Test Method, Item 3; Wood Block Mildew Fungistatic Test Method, Item 4) depending on the nature of the surfaces or articles on which the product is intended to be used. This method is especially applicable for testing products which are applied by non-spray methods of application (wiping, mopping, etc.).

2. Summary of Method.

2.1 This method is conducted using polished cylinders (penicillin cups) as carriers which are seeded with a standardized spore suspension of the test organism. After carriers are dried, they are immersed in the use-dilution of the product, and then placed in test tubes containing culture medium plus fungicide neutralizer. After incubation, evaluation as the presence or absence of fungal growth is made.

3. Apparatus.

3.1 Glassware: Lipless 25 by 150 mm test tubes with cotton plugs. Petri plates 15 by 100 mm with filter paper. Erlenmeyer flasks (250 and 1000 ml) with cotton plugs.

3.2 Racks and Baskets: Suitable for holding test tubes.

3.3 Water Bath: Suitable for maintaining 20°C for the exposure period of the product.

3.4 Carriers: Polished Cylinders (Penicillin Cups), 8 mm type 304, stainless steel. 1/

3.5 Tissue grinder (homogenizer): No. 4288B, Arthur H. Thomas Co.

3.6 Counting chamber: Suitable for determining spore concentration.

4. Reagents and Materials.

4.1 Distilled water or water of equal purity, unless otherwise specified.

4.2 Neopeptone-neutralizer broth: Prepare by dissolving 0.7g lecithin and 5.0g sorbitan monooleate 2/ in 400 ml of hot water and boiling until dissolved. Add 1.0g sodium thiosulfate, 10g, neopeptone, and 20g dextrose. Adjust pH 7.2 ± 0.2 using tris buffer, bring volume to one liter with water and mix thoroughly. Place 10 ml of the medium in cotton-plugged 25 by 150 mm test tubes for sterilization.

4.3 Tris buffer stock solution: 0.1 M. Dissolve 12.1g tris(hydroxymethyl)aminomethane in 500 ml water and bring to one liter. Approximately 70 ml of this buffer solution is used per liter of culture medium. The pH of the medium is approximately 7.2 after autoclaving.

4.4 Sterile distilled water.

4.5 Asparagine solution: stock supply of sterile 0.1% asparagine water solution.

4.6 Sodium hydroxide solution: approximately 1N (4%).

4.7 Saline solution: 0.85% sodium chloride and 0.05% isooctylphenoxypolyethoxyethanol 3/ in distilled water.

5. Test Fungus.

5.1 Aspergillus niger (ATCC 6275) 4/. Maintain stock cultures on neopeptone agar (10g neopeptone, 20g dextrose, 20g agar and 1 liter distilled water). Incubate stock culture 7 days at 25°C then store at 2 to 10°C.

6. Selection of Treatments.

6.1 Test Fungicide: A sufficient number of dosages of the test fungicide should be evaluated in order to determine the minimum effective dosage.

6.2 Untreated Control: Two untreated carriers are included for determining the validity of the test.

6.3 Standard Fungicide: A fungicide registered for use on similar surfaces may be included in the test as a comparative treatment. The product selected should involve a method of application comparable to that of the test fungicide.

7. Procedures. NOTE: Aseptic procedures must be followed during the course of the study.

7.1 Preparation of spore suspension: A spore suspension of fungus is prepared by washing the spores from the surface of 7- to 10-day neopeptone agar culture with sterile saline solution. Spore chains may be broken up by transferring the suspension to a heat-sterilized tissue grinder and reciprocating the piston several times. Filter suspension through a thin layer of sterile cotton or other suitable material to remove spore chains and hyphal elements. Conidial suspensions may be stored at 2 to 10°C for no longer than four weeks. Standardize test conidial suspensions to contain five million conidia per ml by adding sterile diluent. Determine by spore concentration with a counting chamber. 5/

7.2 Preparation of Carriers: Soak metal carriers overnight in 1N sodium hydroxide solution, rinse with tap water several times, then rinse twice with distilled water; place cleaned carriers in multiples of ten in 25 by 150 mm test tubes with closures (or cotton plugs) and cover with asparagine solution. Autoclave at 103 kPa (121°C) for 20 min., cool, and hold at room temperature.

7.3 Inoculation: When the carriers are cooled, pour off the asparagine and cover with standard spore suspension of *A. niger* and allow to stand 10 min. Pour off the spore suspension and remove the 10 carriers to a sterile petri plate matted with filter paper. Allow the carriers to dry at room temperature or in an incubator at 37°C with the petri plate lids ajar. (Note: Drying at room temperature may take up to 12 hours; incubator, 1/2 to 2 hours).

7.4 Treatment: Place 10 ml of the use-dilution of the product in each of ten, 25 by 150 mm test tubes with closures. Place 10 ml of sterile distilled water in each of two, 25 to 150 mm test tubes with closures to serve as viability control (untreated controls). All test tubes are placed in suitable rack and immersed in a water bath to cover the lower 2 in. of the tubes. Allow the tubes to come to temperature equilibrium at 20°C, then place the metal carriers in the test tubes at convenient intervals. When the carriers have been in contact with the product (or water for the untreated controls) for 1 minute, remove them aseptically, allow excess liquid to drain from the carriers and place them individually in 25 to 150 mm test tubes containing 10 ml of neopeptone neutralizer broth. Immediately after placing carriers in test tubes, swirl tubes 3 times. NOTE: Products which are capable of keeping surfaces totally wet for longer than one minute under actual use conditions should be tested under a longer treatment exposure time. To determine the duration of such an increased treatment time the following procedure should be employed. Tests must utilize hard nonporous surfaces (e.g., glass, metal, or porcelain) of at least one square foot in area and which are treated in accordance with the proposed label directions for use. The test surface(s) must be positioned vertically, unless the product is intended solely for use on horizontal surfaces (e.g., floors) in which case horizontal surfaces must be used. The test must be conducted at a temperature of 20 to 25°C and a relative humidity of 50% or less. The length of time (in seconds) from application to when any portion of the treated surface begins to appear dry should be recorded. The average length of this drying time for a minimum of three replicates, shall serve as the basis for determining the increased treatment exposure time. Products which keep surfaces totally wet for longer than 10 minutes should utilize a 10-minute exposure time.

7.5 Incubation: Incubate all tubes at 25°C for at least three days.

8. Determination of Results.

8.1 Evaluation: The presence or absence of fungal growth in the culture medium, after at least 3 days incubation, is the criterion for determining the "cidal" effectiveness of the test product. Fungal growth must be present in both viability control replicates if the test is to be valid.

8.2 Interpretation: A product dosage is considered acceptable when all ten treated replicates are free of fungal growth. The results of this test must be correlated to the intended label claims. Products which pass this test may be labeled as fungicides or mildewcides which kill mold and mildew organisms. If the product is not tested for residual effectiveness, the labeling must state "non-residual" or "kills on contact."

9. Data Reporting.

9.1 Test reports must include all pertinent details of the test conditions and variables. Such information shall include:

9.1.1 Complete description of formulation(s) tested (type of formulation, name and percentage of active ingredient(s), and EPA Registration Number of any standard fungicide used).

9.1.2 Dosage rates (specify whether product or active ingredient, and whether on a weight and/or volume basis).

9.1.3 Effectiveness data--the number of replicates with fungal growth for each treatment being evaluated (including untreated controls).

9.1.4 Modifications--describe the nature of any changes made in the test method and provide the rationale for each change. For example: the duration of any exposure time used which is longer than the 1 minute exposure specified in section 7.4, plus the results of the drying time test used to support the change in exposure time should be reported.

Footnotes

1/ S and L Metal Products Corporation, 58-29 Fifty-seven Drive, Maspeth, N.Y. 11378.

2/ Tween-80, ICI United States, Agricultural Div., Wilmington, Del. 19897.

3/ Triton X-100, Rohm & Haas Co., Phila., Pa. 19104, or other suitable wetting agent such as dioctyl sodium sulfosuccinate, as Aerosol OT solid A-349, Fisher Scientific Co.

4/ Cultures of *A. niger* (ATCC 6275) are available from: American Type Culture Collection, 12301 Parklawn Dr., Rockville, Md. 20852.

5/ For detailed instructions: Tuite, John, 1969. *Plant Pathological Methods, Fungi and Bacteria*, Burgess Publishing Co., Minneapolis, Minn., 1969, pp. 183-184.

II. Fabric, Cordage, and Fibers (Rot, Decay, Mold, and Mildew)

Item 7 - Mildew Resistance of Textile Materials: Soil Burial Method

Federal Test Method Standard. October 5, 1972. Mildew resistance of textile materials; soil burial method. Method 5762.1 in Textile test methods No. 191, General Services Administration, Washington, D.C. 20407.

This test method may be used to support product claims for control of rot and decay as well as mold and mildew of textile materials which are intended for soil contact uses. Products which pass this test may also bear these same claims for uses on textile materials which do not involve soil contact. Rot and decay evaluations should be based on visible deterioration and breaking strength determinations, whereas mold and mildew evaluations should be based on visible fungal growth.

Item 8 - Mildew Resistance of Textile Materials: Mixed Culture Method

Federal Test Method Standard. December 31, 1968. Mildew resistance of textile materials; mixed culture method. Method 5760 in Textile test methods No. 191, General Services Administration, Washington, D.C. 20407.

This method may be used to support claims for control of rot and decay and mold and mildew of textile materials which are not intended for soil contact uses. Rot and decay evaluations should be based on visible deterioration and breaking strength determinations, whereas mold and mildew evaluations should be based on visible fungal growth.

III. Wood (Rot and Decay)

Item 9 - Standard Method for Field Tests with Stakes

American Wood-Preservers' Association. 1969. Standard method for field tests with stakes. AWPA Method M7-69 in AWPA Manual of Recommended Practice.

Separate tests should be conducted for each method of application (pressure, dip, brush, spray, etc.).

Item 10 - Standard Method for Evaluating Wood Preservatives by Field Tests with Stakes

American Society for Testing and Materials. 1974. Standard method of evaluating wood preservatives by field tests with stakes. ASTM Designation: D1758-74.

This method should be used only for pressure methods of application (or equivalent methods which provide the same retention and penetration of preservative).

Item 11 - Standard Method for Field Tests with Posts

American Wood-Preservers' Association. 1950. Standard method for field tests with posts. AWPA Method M8-56 in AWPA Manual of Recommended Practice.

Separate tests should be conducted for each method of application (pressure, dip, brush, spray, etc.)

IV. Paper and Paperboard (Mold and Mildew)

Item 12 - Evaluating Antimycotic Properties of Paper and Paperboard

Vinson, L.J. 1953. The Lever spore cloud method for the evaluation of antimycotic properties of paper and paperboard used in the soap industry. TAPPI 36(5):234-236.

This method may be used for determining the effectiveness of fungicides applied at the dry end of paper and paperboard manufacture. If the paper or paperboard is intended for uses other than the soap industry, the test may be modified by using other organisms.

V. Polymeric Materials (Mold and Mildew)

Item 13 - Resistance of Synthetic Polymeric Materials to Fungi

American Society for Testing and Materials. 1970. Standard Recommended practice for determining resistance of synthetic polymeric materials to fungi. ASTM Designation: G21-75. Amer. Soc. for Testing and Materials, Phila., Pa. 19103

This procedure is limited to materials into which a pesticide is incorporated during the manufacturing process.

VI. Coatings (Mold and Mildew)

Item 14 - Test for Resistance to Growth of Mold on Surfaces of Interior Coatings

American Society for Testing and Materials. 1973. Tentative method of test for resistance to growth of mold on the surface of interior coatings in an environmental chamber. ASTM Designation: D3272-73T. Amer. Soc. for Testing and Materials, Phila., Pa. 19103.

VII. All Crops (Plant Parasitic Nematodes)

Item 15 - Nematicide Test Procedures

The following procedures are to be used for general guidance on planning, conducting, and evaluating nematicide field tests:

American Phytopathological Society and Society of Nematology. 1978. Methods for evaluating plant fungicides, nematicides, and bactericides - Section IV Nematicide test procedures. The American Phytopathological Society, 3304 Pilot Knob Road, St. Paul, Minn. 55121. Pp. 99-134.

American Society for Testing and Materials (Phila., Pa. 19103; 1977):
- Standard guidelines for field evaluation of nematode control agents - Site selection procedures. E 612-77.
- Standard guidelines for evaluation of nematode control agents - Side effects of nematicide applications to other organisms. E 613-77.
- Standard guidelines for field evaluation of nematode control agents - Test materials and environmental conditions. E 628-77.
- Standard guidelines for field evaluation of nematode control agents - Determination of nematode population responses to control agents. E 629-77.

Series 94: EFFICACY OF TERRESTRIAL HERBICIDES, PLANT REGULATORS, DESICCANTS, AND DEFOLIANTS

§ 94-1 Overview.

(a) General. Terrestrial herbicides, plant regulators, defoliants, and desiccants are numerous and extremely diverse in their physical and chemical properties. Moreover, they are applied by various methods under actual use situations and may interact variably with the environment once introduced into the biosphere. These factors directly influence both biological performance (efficacy, and safety to crops and desirable nontarget plants) and residual life (dissipation or persistence) of each pesticide product. Therefore, tests should be conducted to determine the range of conditions within which the product is useful without producing detrimental effects on the crop, on other desirable plant species within the area intended for treatment, or on nontarget plant species in adjoining sites. Testing under actual use conditions is the acceptable means for evaluating the ultimate performance of herbicides, plant regulators, desiccants, and defoliants. Each product should be able to demonstrate its usefulness from applications made according to use directions and under conditions recommended in labeling. Label claims include those expressed, such as weed species controlled, and those implied, such as crop safety and safety to desirable nontarget plant species. Data should encompass the variables expressed on the label, those expected under actual use conditions, and those describing experimental designs, procedures, conditions, and results from supporting tests.

(b) Definitions. The terms herbicide, plant regulator, desiccant, and defoliant are defined in the Act and in § 162.3 of the FIFRA Sec. 3 regulations and are reiterated in §§ 104-2 through -5 of Subdivision H.

(c) Phytotoxicity. Product performance tests with herbicides, plant regulators, defoliants, and desiccants would often also provide concurrent information on phytotoxicity to nontarget plants. Information for determining phytotoxicity are explained in Subdivision J of these guidelines. It is likely that some of the field studies indicated in appropriate sections of this subdivision can be easily combined with studies in by Subdivision J, if necessary.

§ 94-2 General considerations.

(a) Data and test considerations. For complete guidance concerning product performance, §§ 90-1 and 90-2 of this subdivision should also be studied. The product performance recommendations in this section are applicable only to herbicide, plant regulator, desiccant, and defoliant products formulated and packaged for use as pesticides. Data requested for these pesticides should address test conditions which will vary according to desired effects, crops (sites), use conditions, cultural practices, application techniques, and biological activity of the product. Factors to be evaluated and reported should include:

(1) Dosage rates. Dosage rates should be reported in units of active ingredient or acid equivalent as appropriate. Rates may be expressed as units of ingredient per unit of land area to be treated; units of concentration, such as parts per million, applied to obtain a specified degree of foliage coverage, such as runoff; or units of ingredient per unit volume, as in a ripening room. If a product is applied more than once within a year or growing season, each rate and the interval between applications should be indicated. If products are applied in a tank mixture or are applied serially, rates and intervals, as appropriate, should be reported with identification and formulation for each product. Where a range of rates is recommended, labeling should indicate, and data should support, the conditions for which each rate within the range is recommended.

(2) Test location. The state or geographical region where each test is conducted should be reported. The name of the nearest town or city of significant size should be reported.

(3) Statistical analysis. Where preliminary test results such as efficacy, phytotoxicity, commodity quality, or yield, indicate poor or inconsistent efficacy, or adverse effects on crops or commodities, statistical analysis is needed in subsequent tests evaluating the same response(s). As examples, analysis is needed when a plant regulator label claiming yield increase produces inconsistent results (increases and decreases compared to untreated control yields), and when a herbicide decreases crop yields compared to check plots where commercially acceptable levels of weed control are maintained. An additional situation where statistical analysis is useful is in evaluation of interactions resulting from comparability studies supporting tank mixes or serial applications.

(4) Product formulation. To support registration, data should demonstrate usefulness of the exact formulation (product) for which registration is sought.

(5) Application equipment. The application equipment used in testing products in small plots should be designed to simulate grower equipment. This can be readily accomplished by using the basic components of commercial application equipment in the design of the small plot equipment. For example, nozzle types, sizes, and arrangements on small plot sprayers can be identical to those used by growers on commercial ground sprayers; or single-row commercial granular application equipment mounted on a garden tractor for small plot trials should produce results comparable to a multiple of such units on a large tractor. For large-scale trials, commercial application equipment should be used. Specific detail as to descriptions of equipment design, adjustment, and operation should be provided in test reports involving aerial applications and applications using conventional farm equipment (such as tillage or planting equipment), irrigation systems, mechanical incorporation, directed sprays, subsurface placement, or band rather than broadcast distribution.

(i) Aerial application. Aerial applications should include information on dosage, spray volume, maximum height from nozzles to target, type of aircraft (fixed wing or helicopter), ground speed, swath width, nozzle type, orifice size, core, nozzle pressure, nozzle discharge orientation, number and arrangement of nozzles, adjuvants used, carrier used, wind velocity and direction, air stability, air temperature, and relative humidity. See § 126-1 through -4 for additional data guidance. Since some of the label-recommended measures, limitations and/or restrictions proposed to reduce drift can, by altering droplet size and distribution (coverage), decrease efficacy and/or increase crop injury in the target area, field efficacy and crop phytotoxicity data (see Subdivision J) should be developed in support of using the application equipment, equipment adjustment and operation, procedures and conditions as allowed or recommended in labeling to reduce drift. Although it is preferable to evaluate swath displacement and performance on the same or an adjacent site, it is not mandatory. Regardless of the site for this performance evaluation, the equipment, equipment operation and adjustment, and procedures should be documented in detail. Aerial application performance evaluation should be run in conjunction with the spray drift evaluation study described in Subdivision R. Application conditions should be documented and should be very similar to those found in the spray drift evaluation study.

(ii) Irrigation system application. Data on efficacy and on crop phytotoxicity, yield, and quality should be developed as in conventional herbicide or plant regulator applications. In the case of irrigation system applications, however, multiple plots within a treated field should be examined for efficacy, crop phytotoxicity, yield, and quality of harvestable commodity as an indication of pesticide distribution. Data from such plots should be reported for each individual plot and not simply averaged together. Further, information on effects of the product(s) on injection pumps (metering devices), gaskets, fittings, hoses, and nozzles, with special attention to wear, clogging, and deterioration, should be provided if requested. It is very important that, in addition to the standard data requirements for conventional applications, data should cover soil texture, percent soil organic matter, relative soil moisture condition (dry, medium, wet) at application, acre-inches of water applied, and precipitation quantities within one week after application.

(A) For sprinkler (overhead) irrigation systems, plots should be located at several nozzle positions along the length of the lateral. Plots should be placed at both extreme ends of the lateral as well as in areas where sprinkler patterns overlap. Comparisons should be made between adjacent plots treated conventionally with ground application equipment and irrigated with untreated water, and plots treated through the irrigation system.

(B) For surface irrigation systems (flood and furrow), plots should be located at the points where the treated water enters the field and at the lower end of the field. Where furrow irrigation is used, data should indicate the spatial relationship between crop rows and furrows. If weed control in furrow irrigation applications is intended only for the furrow itself and not the bed between the furrows, the data should so indicate.

(iii) Directed sprays. When sprays are directed towards or away from certain portions of the soil or plants, data should indicate nozzle arrangements, nozzle orientations, and the extent of spray contact with soil or plants.

(iv) Subsurface soil application. When herbicides or plant regulators are applied directly beneath the soil surface, test reports should include information on equipment. For example, for injection equipment, the following must be specified: nozzle spacing, depth of operation, nozzle pressure, speed of operation, and volume of liquid or gas applied per unit area.

(6) Timing of applications. Where crop plants are or will be involved in the application of any herbicide, plant regulator, desiccant or defoliant, their stage of growth or development at application should be described in test reports and labeling. Test data supporting products intended for use as herbicides should indicate the stage of weed growth at application. If weeds have emerged, growth stage should be indicated in terms of the numbers and/or types of leaves, weed height, or stage of development such as "prior to flowering". Reports involving herbicide applications to emerged weeds should indicate those instances where weed growth rates detrimentally affect product efficacy.

(7) Meteorological conditions. Where product efficacy and/or adverse effects are dependent upon or affected by meteorological conditions, the specific factor(s), such as temperature, wind conditions, precipitation, or daylength, affecting product activity should be measured and reported. Edaphic factors, such as relative soil moisture conditions (dry, medium, wet) and temperature, which are directly affected by meteorological conditions, should also be measured and reported when such factors affect product efficacy and/or adverse effects.

(8) Spray dilution. In certain foliage applications, the concentration or dilution of product in the finished spray and volume of finished spray applied per leaf surface area or per plant are more closely related to product activity levels than the quantity of product applied per unit land area. As an example, a consistent plant response from a given concentration of plant regulator applied to the point of foliage runoff in a fruit orchard may require from 50 to 500 gallons (189.2 to 1892.5 liters) of equally diluted finished spray per acre (hectare), depending upon populations and sizes of the trees involved. In cases such as this, data should include volume of finished spray applied per unit land area, plant population (number of trees per unit land area), age of the planting, concentration of product in the spray solution, and the extent of foliage coverage (such as volume of finished spray per tree or application to the point of runoff). Regardless of the presence or absence of foliage at application, performance data should support the range of spray volumes and spray dilutions recommended in the label use directions.

(9) Adjuvants. Products with labeling which allows or recommends the additions of separately packaged adjuvants to the spray tank should be supported with data indicating their benefits (if claimed) and any detrimental effects (such as increased crop phytotoxicity) which may result from their addition to the herbicide, plant regulator, desiccant, or defoliant. The only adjuvants actually permitted for use with a pesticide will be those adjuvant brand names or defined adjuvant classes specifically named on the pesticide label. The adjuvant rate or range of rates should be

indicated on the pesticide label, and should be supported with data on efficacy and any detrimental effects. If a range of adjuvant rates is recommended, the maximum and minimum rates within that range should be evaluated in conjunction with the intended pesticide product.

(i) Adjuvants claiming increased pesticide product efficacy. If claims are made or implied on pesticide product labels for increased efficacy from the pesticide adjuvant combination over use of the pesticide alone, such claims should be supported with efficacy comparability data from adjacent plots where the pesticide is applied with and without the adjuvant and where the comparison is analyzed statistically.

(ii) Adjuvants without pesticide efficacy claims. If adding an adjuvant to the pesticide product or spray mixture is mentioned on the label of the pesticide product, but no claims are made for increased performance, then crop effects data should be developed to support maintained performance and no increase in detrimental effects. Efficacy comparability data from adjacent plots (with and without the adjuvant) should be developed as described in paragraph (a)(9)(i) of this section.

(iii) Adjuvants essential to pesticide efficacy. If the label of a pesticide product indicates that an adjuvant must be used in addition to the pesticide, field efficacy data [as in paragraph (a)(9)(i) of this section] should be presented which substantiates this essentiality, since such a statement is an implied efficacy claim.

(10) Untreated control (check) plots. In efficacy evaluations, all treated plots, plants, and/or commodities should be compared directly to untreated control plots, plants, and/or commodities. All quality and/or yield evaluations of plant regulator, desiccant, and/or defoliant treated plants or commodities should be compared to control plants or commodities receiving the same pesticides (herbicides, insecticides, fungicides) except the one being evaluated. Detailed descriptions of plots and plants used as control treatments for comparisons of efficacy and detrimental side effects should be included for each test. Data should indicate if the control plot or plants were untreated (received no herbicide, plant regulator, desiccant or defoliant), received standard cultural practices (which should be described), and received other pesticides (which should be listed). Data from untreated plots or plants should include measurements of the same factors to be evaluated in determining efficacy and detrimental side effects resulting from the proposed herbicide, plant regulator, desiccant, or defoliant application. For example, data from untreated weedy control plots for a herbicide should include weed species present, relative weed populations (density), and weed vigor; data from tests with a plant regulator such as a fruit ripener should include, for example, fruit coloration, sugar/acid ratio, yield, quality, and dollar value per unit for untreated fruit. If, in addition to the untreated control plots, plants, and/or commodities, a registered product is also applied (as a standard) for comparison of efficacy and/or detrimental effects, data should indicate the standard product's name, active ingredient, dosage rate, and performance results.

(11) Tank mixture compatibility. Herbicides, plant regulators, desiccants, or defoliants along with other pesticides, recommended carriers, and adjuvants which are to be mixed together in the same tank for simultaneous

application should be physically compatible to allow uniform and useful application. While tests conducted under actual use conditions should support performance and compatibility of such mixes, laboratory tests should be conducted to evaluate physical compatibility of maximum rates of all components of the tank mix in the minimum carrier volume recommended per unit area. [See also § 94-3(b)(6).] When adjuvants added to increase compatibility are recommended or allowed, effects of the minimum recommended rates of these adjuvants on tank mixture compatibility should be evaluated. Data should indicate the order of addition of components to the test container, field equivalency rates per unit area for each component, and the field equivalency spray volume per unit area. Data should also indicate the extent of agitation used in the tests. It is important, where components are in some degree incompatible in static tests, that effects of agitation and sprayability be evaluated and reported.

(i) Laboratory tests for water carrier. If the carrier is limited to water, tests should evaluate effects of water temperature, pH, and hardness of compatibility, sprayability (using conventional screens, nozzles, and pressures), and resuspendability. Water temperatures tested should include ranges between 4°C and 32°C, pH levels tested should include 6, 7, 8, and 10, and hardness tested should include both soft water (less than 15 ppm calcium carbonate) and hard water (greater than 200 ppm calcium carbonate). Resuspendability should be evaluated after 24 hours without agitation. If any of these variables are found to markedly reduce compatability, label instructions should include appropriate limitations.

(ii) Laboratory tests for fluid fertilizer carrier. If fluid fertilizers are used as partial or total carriers, laboratory tests should evaluate compatibility, sprayability, and resuspendability using major classes of fluid fertilizers, such as high nitrogen and complete analysis, and suspensions. Laboratory tests are of value in that compatibility problems with major classes of fluid fertilizers can be detected and addressed through label cautions. Due to the larger number of possible fertilizer sources and analyses, laboratory tests may involve only major classes of fluid fertilizers, and emphasis should be directed, via the label, towards use of a "jar test" by the applicator prior to large scale mixing. Employing the exact fertilizer source and analysis intended for application in a jar test with maximum rates of all other components is the only fully reliable method for determining compatibility.

(12) Cultural practices. Cultural practices for a given crop vary with production areas and between growers within an area. The effects of cultural practices on the product's usefulness should, therefore, be addressed.

(i) Irrigation. Irrigation and watering practices should be studied as a variable if the product is to be used in irrigated areas or greenhouses, respectively. The influence of different irrigation practices should be studied in the use area. Irrigation data should include a description of equipment and techniques used in water application, the number and timing of irrigations, and quantity of water in acre-inches (hectare-centimeters) applied at each irrigation. Also, description of the chronological relationship between irrigation applications and nerbicide, plant regulator, desiccant and/or defoliant application is needed. Where flood irrigation is utilized (such as in rice production), depth, duration, and any "flushing" should be described for each test. When irrigation is used to activate a pesticide product in the absence of precipitation, the maximum application-to-irrigation interval (producing the desired efficacy level) should be supported by data. When irrigation is allowed or recommended (in labeling) just after pesticide application, the minimum application-to-irrigation interval (not detrimentally affecting pesticide performance) should be supported by data. Since crop safety is often influenced by pesticide placement in the soil profile, and irrigation may directly affect such placement, label-recommended or -allowed irrigation practices should be supported by crop safety data (phytotoxicity and yield). When irrigation practices result in loss of pesticide-contaminated water (as in runoff or drainage) from the target area, data should address effects of such water on nontarget plants.

(ii) Mowing. Mowing operations may have a direct effect on efficacy or detrimental effects of herbicides and plant regulators intended for use on lawns, turf, golf courses, median strips, pastures, rangeland, and hay and forage crops. Lush foliage may reduce efficacy by intercepting products which are soil active, or maturing weed seedheads may constitute a source of reinfestation even after herbicide application. Plant regulators and herbicides which are dependent upon foliage contact for translocation may require lush unmowed foliage for effectiveness. Mowing just prior to or just after herbicide applications may, by mechanically injuring desirable plants or by decreasing growth rates, increase injury to desirable plants from the interaction of mechanical and chemical effects. Mowing just prior to application may be a requirement for plant regulators intended to maintain the neat appearance of grassy areas by retarding grass growth. In situations where mowing is routinely a part of cultural practices, or may influence efficacy or detrimental effects, such practices should be reported in test results, and, where appropriate, should be accounted for in use directions and/or cautions on product labeling.

(b) Comparability tests. (1) In certain circumstances, the registrant may have the option of establishing comparability of performance between proposed and registered uses of formulations rather than conducting a large-scale testing program in which all variables are again extensively reevaluated pertinent to the amended use or altered formulation. These circumstances may apply when a registrant desires to amend a registered label by altering directions for use, rates, or application techniques, or by adding tank mixes, serial applications, adjuvants, or alternate carriers, or if a registrant desires initial registration of an alternate formulation of a product which has been previously registered. Use of this approach will generally decrease the time and expense involved in generating performance data. Prior to initiating this alternate approach, discussions with Agency personnel should be held to determine the propriety of this approach and the performance data encountered in the specific situation. Establishing comparability should direct comparisons of results from plots (preferably adjacent) subjected to identical test conditions.

(2) Since tests may be fewer in number and smaller in size than those involved in full scale testing programs, more emphasis should be placed

on selection of test conditions such that comparability will be evaluated under the most rigorous test conditions. As an example of rigorous conditions for a herbicide, plots should be selected which are likely to contain the more resistant weed species of those claimed on the label.

(c) Data guidance for manufacturing-use products. Data are recommended for certain of the "manufacturing-use" products, including those pesticides intended for formulation of other pesticidal products, and those pesticides intended for repackaging. In support of those pesticides intended for reformulation, proof of pesticidal usefulness is needed, applicants may either develop usefulness data [as to the type of pesticide(s) named] based upon presumptive laboratory data and include a label statement notifying formulators of their responsibility for providing supporting data for their formulations, or have usefulness data for each product formulation and include sample labeling for each formulation. For those pesticides intended for repackaging, in-use performance data which support the registered label of an identical formulation may be referenced. If additional uses of formulations other than those on the registered label of the identical formulation are sought, then additional usefulness data should be developed.

(d) Usefulness. Usefulness of herbicide, plant regulator, desiccant, or defoliant products includes both efficacy and any detrimental side effects to crops or harvestable commodities, and adverse effects to nontarget plants. For each of the above types of products, efficacy requirements and detrimental effect evaluation considerations are discussed in §§ 94-3 in terms of specific use patterns.

§ 94-3 Terrestrial herbicides.

(a) General considerations for herbicides. Efficacy data should support each weed species claimed on the label. See § 104-2(a)(3)(i) for appropriate weed nomenclature. Only when data support consistent broad spectrum control of weeds should the use of general claims such as "existing vegetation", or "annual weeds" be used on labels. Efficacy data should support control of each species at each minimum rate recommended for a particular situation. As an example, if recommended rates vary with soil textures (such as coarse, medium, and fine), then data should support weed control by the minimum recommended rate for each of the three textural groups. If rates are further defined for ranges of soil organic matter content within a particular soil texture, weed control data should support control of species at the minimum rate recommended for that specific situation. If rates depend on conditions defined in labeling use directions or in limitations, such as soil texture, weed species, weed height, weed density, crop height, or other measurable conditions, then supporting data should include an assessment of these conditions so that use of recommended rates can be verified. Tests using excessive rates for a particular situation may be of value in evaluating crop tolerance if a crop is involved; however, use of test rates above those recommended by labeling for a particular situation are of no value in supporting efficacy claims. Use of rates proving to be efficacious, but lower than those recommended for a particular situation, may be considered in evaluating efficacy. Weed control from herbicide applications should be expressed as a comparison with an untreated control. Except for the herbicide application, both treated and untreated plots should receive similar cultural practices. Methods of measuring the weed control achieved can take the form of either visual observations by experienced personnel or actual weed counts per given area (stand) and/or measurement of weed height, vigor, or weed weight. Control may be indicated as either percent control or as a rating. Percent control can be calculated by the formula: weed growth in untreated control plot less weed growth in treated plot, divided by weed growth in untreated control plot x 100 = percent weed control. If a rating other than percent control is desired, some other rating scale can be used, such as zero representing no weed control (equal to untreated control) and ten equalling 100% weed control. Where a rating scale other than percentage is used, an explanation of the scale which indicates the levels representing no control, partial control, commercially-acceptable control, and perfect control should be included in the report.

(1) Suggested performance standard. In order to justify a label claim for weed control, the degree of control in supporting data need not be perfect (100%), but should consistently reach a level which is considered commercially acceptable. Commercially acceptable levels may vary slightly from one use pattern and weed problem to the next; however, the acceptable level should, in all cases, be expected to provide a meaningful benefit to the ultimate user of the product. For the claim of "control", the product usually must provide at least the equivalent of 70% reduction in weed stand and/or vigor when compared to untreated check plots under moderate to severe weed pressures. A lesser label claim for "suppression" or "partial control" of certain weed species may be appropriate on labels containing valid claims for "control" of other weed species. The terms "suppression" and "partial control" may indicate either consistent weed stand and/or vigor reductions at levels considered less than commercially acceptable, or inconsistent levels of weed stand and/or vigor reduction. Even though considered a lesser claim than "control", claims for "suppression" and "partial control" should be supported by efficacy data which demonstrates a benefit. A herbicide label bearing claims for only "suppression" and "partial control" of weed species, without any accompanying claims for "control" of other weed species, may be considered inappropriate for registration depending upon the demonstrated level of benefit to the user. Combined claims using wording such as "control or suppression" are not acceptable. If certain conditions or rates allow only "suppression" or "partial control" of weed species while other conditions or rates allow "control" of the same species, these conditions or rates should be identified in labeling and be associated with the appropriate level of response reflected in the supporting data.

(2) Reporting of data. The degree of control of each weed species in a test should be reported on a species-by-species basis. Since weed species vary in their susceptibility to specific herbicides, and since label claims are made on a species-by-species basis, reporting only broadleaf and grass control or reporting a single percent control with a list of weeds present is not considered sufficiently detailed to support specific label claims. In addition to individual weed control ratings, information is needed which indicates weed pressure by species in the untreated check plots.

Such data should include both weed populations and vigor. In addition to weed control data, secondary benefits derived from weed control may in certain cases be supportive data. Depending upon the objective of weed control in a particular use pattern, supportive data may include such evaluations as decreased trash at harvest, increased harvest efficiency, increased water retention from chemical fallow, or increased carrying capacity from renovated rangelands. In no case should such supporting data be considered solely as evidence of efficacy in lieu of data on herbicidal effectiveness.

(b) Specific data considerations. In addition to the general herbicide considerations, the following specific use patterns should have additional data as indicated.

(1) Soil incorporated products. For herbicides intended to be soil incorporated, data and labeling should address application-to-incorporation intervals, and intervals between incorporations (if repeated). Data should include identification and/or description of incorporation equipment, depth of equipment operation, and depth of incorporation of the pesticide.

(2) Systemic herbicides applied to foliage. The quantity of weed foliage (expressed in terms of weed height or development) provided for herbicidal activity should be reported in performance data and indicated in product labeling. Further, where systemic foliage applications are intended to control roots or other underground plant parts, performance data should address (and product labeling should indicate) a minimum application-to-tillage interval which will allow sufficient time for translocation and herbicidal effects to take place.

(3) Seedbed preparations for preplant and preemergence (to crop) applications. Selected methods of seedbed preparation common to the test crop should be studied and reported. It is suggested that such methods cover the extremes from poorly- to well-prepared seedbeds to study effects of clods and plant debris on product efficacy and possible crop injury. These extremes should be within the limits normally encountered by growers. If seedbed preparation does not affect weed control or crop injury in preliminary trials, the reporting of seedbed preparation in subsequent trials may not be critical.

(4) Cultivation practices. Cultivation practices should be those common for the crop in the test site. Since many crops are being grown under minimum tillage practices, it may be desirable to determine the effectiveness, both initial and residual, of the herbicide without cultivation. If cultivation is required to supplement the product's usefulness, subsequent trials should be designed to show the effects of the product used without cultivation, the effects of cultivation only, and their combined effects. Some cultivation practices may reduce the effectiveness of the product through movement of treated and/or untreated soil; these should be recognized and studied as variables. When cultivation is allowed or recommended, or when it affects product usefulness, cultivation practices should be addressed in test reports and labeling. Weed control ratings recorded after cultivation should be so indicated.

(5) Herbicide use with fertilizers. (i) Fertilizer practices should be within the range recommended for the crop in the test location; however, where herbicides are tank-mixed with fluid fertilizers or are impregnated onto dry fertilizers, specific information is needed. As to information on fluid fertilizer/herbicide tank mix applications, field test data should indicate spray volume per unit area, fertilizer analysis, compatibility, efficacy, and detrimental effects. For additional considerations on pesticide/fluid fertilizer tank mixes, see §§ 90-2(b)(4) and 94-2(a)(11)(ii).

(ii) Reports of data supporting use of herbicide impregnated dry fertilizers should include, in addition to efficacy and detrimental effects, the quantity of fertilizer applied per unit area, the fertilizer analysis, the quantity of product impregnated onto a ton of fertilizer, and the tillage (soil incorporation) operations. In addition, see § 104-2(c)(3) and (4).

(6) Tank mixtures. (i) Tank mixes containing herbicides are viewed as new products, regardless of the history of individual components, for which all label claims should be supported. Claims for a tank mix should be indicated on the label of at least one of the registered components. Refer also to § 164-4 of Subdivision N.

(ii) Claims may be made by: listing the specific weed species supported by data from actual testing of the proposed mix; claiming all weeds from the labels of each product involved in the mix and supporting these claims with appropriately referenced efficacy data and performance comparability data which indicate the extent of antagonism and synergism between the products; or combining these approaches when a label claims all weeds from the labels of each product involved in the mix and claims additional weed species not listed on any of the component product labels.

(iii) When tank mix labeling claims all weed species from labels of each product involved in the mix, but supporting data for each species have not been generated from use of the proposed mix, either the claims should be restricted to those actually supported by tank mix data, or performance comparability plots should be established and appropriate efficacy data supporting the claims for each component should be referenced. Comparability tests should demonstrate any change in efficacy or detrimental effects due to antagonistic or synergistic effects of each product on the other(s). It is not required that each weed species to be claimed for the tank mix be present in comparability test plots, but rather such tests include those claimed species considered most difficult to control. Antagonism and synergism are best evaluated in adjacent replicated plots where possible interactions are subjected to statistical analysis.

(iv) For conventional tillage practices, adjacent plots should compare usefulness of each product alone (at appropriate rates) with usefulness of the proposed tank mix.

(v) When products which are tank-mixed have divergent modes of action, such as a minimum tillage tank mix containing a contact herbicide for emerged vegetation control combined with a residual herbicide for preemergent (to weeds) weed control, observations of weed control should be timed such that contact and preemergent activities are reported separately. In situations where both contact and preemergent products are tank-mixed or applied serially for minimum tillage weed control, it is relatively simple to claim only those weeds directly supported by tank mix data; however, when preemergence weed control is to be supported by comparability data, evaluation of interactions between the two types of products may be more complex. Effects of a preemergent (to weeds) herbicide on usefulness of a contact product can be evaluated in adjacent minimum tillage plots comparing contact product activity with and without the preemergent product. Effects of the contact chemical on preemergence activity under minimum tillage conditions is practically impossible to evaluate due to the existing vegetation cover. Since effects of the contact chemical on preemergence activity should be evaluated without interference from the existing vegetation, and herbicidal control of existing vegetation would not resolve questions of antagonism or synergism, possible interaction of the contact product on emergence activity should be evaluated in adjacent conventionally-tilled plots. In the conventionally-tilled plots, where existing vegetation has been mechanically turned under the soil surface, the preemergence product should be evaluated with and without the contact product.

(vi) Data in support of two or more herbicide products mixed partially or completely in fluid fertilizers should either support all label usefulness claims using the fluid fertilizer as carrier, or establish performance comparability with water as the carrier in adjacent plots.

(7) Serial applications. Data considerations for serial application(s) of one or more herbicide(s) preceding or following another herbicide on the same crop area in the same growing season are identical to those described for tank mixes, except that the times of application (or application interval) should be indicated for each product or tank mix involved in the serial application. Physical compatibility data as for components of tank mixes are not required for serial applications. [See also § 90-2(a)(3) and §164-4 of Subivision N for criteria on serial applications.]

(8) Noncrop area applications. Use sites, such as rights-of-way, lumberyards, or fence rows, should be indicated in test reports and labeling. If claims are made for longevity of weed control, such claims should be supported with efficacy data. Where bare ground is desired (for example, for fire prevention in lumber yards), the following should be reported: weed escapes, duration of weed control, the percent bare ground before and after application, identification, dosages and dates of previous herbicide applications, and the extent of weed reinvasion from untreated areas.

(9) Chemical fallow applications. Labels and performance data supporting products for use in chemical fallow programs should address the longevity of weed control expected, the extent of mechanical tillage required to supplement herbicide activity, the extent of mechanical tillage allowed which will not decrease herbicide activity, and scheduling of harvest, application, tillage, fallow, and replanting appropriate for the cultural practices followed in the geographical area for which use of the product is intended.

§ 94-4 Plant regulators.

(a) Scope. Plant regulators include but are not limited to products that increase yields, increase flowering, thin flowers and fruits, increase fruitset, decrease fruit drop, accelerate ripening, concentrate sugar content, enhance abscission, chemically "pinch" ornamentals, reduce internode elongation, inhibit tobacco sucker growth, and promote rooting of cuttings. Data supporting efficacy claims and freedom from adverse effects on crop plants from plant regulators are unique for each use pattern and crop. Often, varying concentrations or varying applications of a product to a single plant species at different growth stages will cause a wide variety of responses. Many plant regulators eliciting a response in one variety or cultivar of a species will not perform similarly at all on another variety within the same species. Further, many of the plant response levels are extremely subtle and at times may be inconsistent within the same closely controlled tests.

(b) Suggested performance standard. Efficacy data in support of plant regulator claims need not demonstrate perfect (100%) plant response, but the response attained should be at a level considered commercially acceptable. Commercially acceptable plant response levels vary from one use pattern, plant species or variety, and environmental condition to another; however, the response level should, in all cases, be expected to provide a meaningful benefit to the user of the product.

(c) Suggested performance reliability. Due to the frequency of subtle and inconsistent responses of plants or commodities to plant regulators, efficacy evaluations should be replicated and statistically analyzed where greenhouse or small plot tests are conducted. When commercial application equipment is utilized in large field plots where independently replicated applications may not be practical or economical, efficacy evaluations should be based upon multiple independent observations and/or samples taken from within each treated area. As the response levels approach the limits of usefulness, increased replication and sampling are needed to support efficacy claims and absence of significant undesired effects.

(d) Efficacy and adverse effects data. The following data should be considered in addition to the general considerations in § 94-2. The untreated control crops or commodities should receive the same cultural practices, post-harvest treatment, and pesticide applications (excluding the product being evaluated) as the treated plants or commodities. Due to the varied responses, sites, crops involved, use patterns, and application techniques (all frequently involving the same product), each product is evaluated by the Agency on a case-by-case basis and according to claims made on the specific product label for the specified crop or commodity. For example, a ripener for use on tomatoes, tobacco, and pineapples should be evaluated using different criteria for each crop. As a result, a document detailing the criteria for all plant regulator uses on all crops or commodities would be enormously extensive. Therefore, the data considerations which follow are not intended to serve as an exhaustive list nor to exclude other plant regulator uses, but instead to include only general guidance which may be applied to certain categories of plant regulator uses. See Subdivision J for a full explanation on hazard evaluation to nontarget plants.

(e) Specific data considerations. (1) Products increasing yields. (i) Comparisons should be made between the treated crop (and/or commodity) and the untreated crop (and/or commodity) with respect to:

(A) Crop or commodity yield per unit area;

(B) Crop stand and vigor;

(C) Crop or commodity quality (grade, dollar value, flavor, size, shape, color); and

(D) Observations on plant growth, flowering, fruitset, yields, and plant or commodity quality obtained during the second growing season after initial application (if the product is intended for yearly or more frequent use on perennial plants).

(ii) The following should also be reported: crop identification as to species and variety, and (as applicable) cultivar, rootstock, or parentage; stage of crop or commodity development at application and at harvest; application-to-harvest interval; harvest technique employed; and any other pesticides applied to the crop or commodity in the same growing season.

(2) Products increasing flowering. (i) Comparisons should be made between treated plants (and/or commodities) and untreated plants (and/or commodities) with respect to:

(A) Number of flowers per plant;

(B) Flower quality (size, shape, color);

(C) Timing of full bloom after treatment;

(D) Growth rate, and conformation of vegetative plant parts;

(E) Data as in paragraph (d)(1) of this section, if increased flowering is intended ultimately to increase yields; and

(F) Observations on plant growth, flowering, fruitset, yields, and plant or commodity quality obtained during the second growing season after initial application (if the product is intended for yearly or more frequent use on perennial plants).

(ii) The following should also be reported: detailed crop identification, stage of plant growth at application, application-to-harvest interval, and any other pesticides applied to the crop or commodity in the same growing season.

(3) Products thinning flowers and/or fruit. (i) Comparisons should be made between treated plants (and/or commodities) and untreated plants (and/or commodities) with respect to:

(A) Percent flower drop and/or fruit drop per plant:

(B) Effects on remaining flowers and/or fruit, conformation of vegetative growth, and phytotoxicity;

(C) Data as in paragraph (d)(1) of this section, if thinning of flowers and/or fruit is intended ultimately to increase crop or commodity yields and/or quality; and

(D) Observations on plant growth, flowering, fruitset, yields, and plant or commodity quality obtained during the second growing season after initial application (if the product is intended for yearly or more frequent use on perennial plants).

(ii) The following should also be reported: stage of plant growth or development at application, detailed plant identification; and any other pesticides applied to the crop or commodity in the same growing season.

(4) Products increasing fruitset. (i) Comparisons should be made between treated plants (and/or commodities) and untreated plants (and/or commodities) with respect to:

(A) Number of fruit set per plant;

(B) Fruit quality (grade, size, shape, color, flavor, dollar value;

(C) Data as in paragraph (d)(1) of this section if increased fruitset is intended ultimately to increase crop or commodity yields and/or quality;

(D) Number of successful crosses and number of seeds per cross, if increased fruitset is intended ultimately to enhance plant breeding; and

(E) Observations on plant growth, flowering, fruitset, yields, and plant or commodity quality obtained during the growing season after initial application (if the product is intended for yearly or more frequent use on perennial plants).

(ii) The following should also be reported: state of plant growth or development at application, detailed plant identification, and any other pesticides applied to the crop or commodity in the same growing season.

(5) Products decreasing preharvest fruit drop. (i) Comparisons should be made between treated plants and/or commodities and the untreated plants and/or commodities with respect to:

(A) Percent fruit drop or retention;

(B) Fruit quality (grade, size, shape, color, flavor, dollar value), and storage and shelf life characteristics;

(C) Harvest date;

(D) Effects on plant growth, immature fruit, yield; and

(E) Observations on plant growth, flowering, fruitset, yield, and plant or commodity quality obtained during the second growing season after initial application (if the product is intended for yearly or more frequent use on perennial plants).

(ii) The following should also be reported: application-to-harvest interval, stage of fruit growth or maturity at application, detailed plant identity, duration of effectiveness, harvest technique, harvest efficiency, and any other pesticides applied to the crop or commodity in the same growing season.

(6) Products accelerating ripening. (i) Comparisons should be made between treated plants (and/or commodities) and untreated plants (and/or commodities) with respect to:

(A) Sucrose content;

(B) Sugar/acid ratio;

(C) Internal and external coloration;

(D) Quality (grade, size, shape, firmness, texture, flavor, dollar value);

(E) Effects on plant or commodity yield, rate of maturation, storage and shelf life characteristics, and harvest efficiency; and

(F) Observations on plant growth, flowering, fruitset, yields, and plant and commodity quality obtained during the second growing season after initial application (if the product is intended for yearly or more frequent use on perennial plants).

(ii) The following should also be reported: application-to-harvest interval, detailed plant identification, plant or commodity stage of growth or maturity at application, and any other pesticides applied to the crop or commodity in the same growing season.

(iii) Refer to § 94-30 paragraph II.G. for additional guidance.

(7) Products concentrating or preserving sugar content. (i) Comparisons should be made between treated plants (and/or commodities) and untreated plants (and/or commodities) with respect to:

(A) Soluble solid content of expressed juice;

(B) Sucrose purity;

(C) Sucrose yield per unit land area;

(D) Effects on plant and commodity yield, quality, rate of maturation, harvest efficiency, and storage and shelf life characteristics; and

(E) Observations on plant growth, flowering, fruitset, yields and plant and/or commodity quality obtained during the second growing season after initial application (if the product is intended for yearly or more frequent use on perennial plants).

(ii) The following should also be reported: stage of plant growth or maturity at application, application-to-harvest interval, detailed plant identification, duration of effectiveness, plant stand, age of planting, and any other pesticides applied to the crop or commodity in the same growing season.

(8) Products enhancing fruit abscission. (i) Comparisons should be made between treated plants (and/or commodities) and untreated plants (and/or commodities) with respect to:

(A) Pull force for fruit removal;

(B) Fruit quality (grade, size, shape, color, flavor, dollar value);

(C) Harvest efficiency;

(D) Effect on fruit and leaf drop, and yield; and

(E) Observations on plant growth, flowering, fruitset, yields, and plant or commodity quality obtained during the second growing season after initial application (if the product is intended for yearly or more frequent use on perennial plants).

(ii) The following should also be reported: application-to-harvest interval, stage of plant growth or maturity at application, harvest technique, ultimate fate of fruit (fresh market or processing), detailed plant identification, and any other pesticides applied to the crop or commodity in the same growing season.

(iii) Refer to § 94-30 paragraph II.H. for additional guidance.

(9) Products chemically pinching ornamentals. (i) Comparisons should be made between treated plants and untreated plants with respect to:

(A) Number and quality of buds, flowers, and lateral branches;

(B) Plant conformation;

(C) Effects on flowering cycle, usability for cuttings, and growth rate; and

(D) Observations on plant growth, flowering, and plant quality obtained during the second growing season after initial application (if the product is intended for yearly or more frequent use on perennial plants).

(ii) The following should also be reported: application-to-harvest interval, plant stage of growth or development at application, detailed plant identification, and any other pesticides applied to the crop in the same growing season.

(10) Products reducing plant internode elongation. (i) Comparisons should be made between treated plants and untreated plants with respect to:

(A) Length of internodes;

(B) Plant size and conformation;

(C) Effects on growth rate, flowering, fruitset, yield, crop or commodity quality, and harvest efficiency; and

(D) Observations on plant growth, phytotoxicity, flowering, fruitset, yield and plant or commodity quality obtained during the second growing season after initial application (if the product is intended for yearly or more frequent use on perennial plants).

(ii) The following should also be reported: stage of growth at application, application-to-harvest interval, duration of effectiveness, detailed plant identification, and any other pesticide applied to the crop in the same growing season.

(11) Products decreasing tobacco sucker growth. (i) Comparisons should be made between treated plants (and/or commodities) and handsuckered and topped-but-not-suckered untreated plants (and/or commodities) with respect to:

(A) Number and fresh weight of suckers at harvest;

(B) Effects on leaf drop, yield, and cured leaf quality (percent usable by tobacco companies, chemical and physical properties, and smoke taste test); and

(ii) The following should also be reported: detailed plant identification, stage of plant growth or sucker development at application, and any other pesticides applied to the crop in the same growing season.

(iii) Refer to § 94-30 paragraph II.A. for additional guidance.

(12) Products promoting rooting of cuttings. (i) Comparisons should be made between treated cuttings and untreated cuttings with respect to:

(A) Number or weight of roots per cutting and/or percent of rooted cuttings per treatment;

(B) Effects on growth rate and conformation; and

(ii) The following should also be reported: type of cutting (stem, root, leaf, softwood, herbaceous, greenwood, or hardwood), rooting medium, detailed plant identification, application-to-observation interval, and any other pesticides applied to the plant in the same growing season or to the cuttings during the period of observation.

(13) Products inhibiting Irish potato tuber sprouting. (i) Comparisons should be made between treated tubers (or tubers from treated plants) and untreated tubers (or tubers from untreated plants) with respect to the factors listed below:

(A) Sprouting (sprout number or weight per tuber), tuber weight loss and rot loss, tuber appearance (flattening, shriveling, discoloration, skin texture), suberin and wound periderm formation, heat generated in piled potatoes, quality (sugar content, mealiness, fry color, flavor, chipping) both before and after storage, usefulness of tubers as propagation stock, shelf life (at time and marketing); and

(B) Yield, and grades, if the plant regulator is applied preharvest.

(ii) The following should also be reported: storage conditions (temperatures, humidity, air circulation, and duration); stage of plant and/or tuber growth or maturity at application; environmental factors affecting usefulness if plant regulator is applied prior to harvest; rates and repeat treatments applied; other pesticides applied to plants and tubers; duration of exposure to sprout inhibitor; crop variety; length of growing season; application equipment; finished spray volume per unit area or per unit measure of tubers; or volume or weight of gas or liquid per unit volume of storage space.

(iii) Refer to § 94-30 paragraph II.B. for additional guidance.

(14) Products increasing branch angle in young nonbearing fruit trees. (i) Comparisons should be made between treated plants and untreated plants with respect to:

(A) Branch angle (angle less than 90° formed by the main truck and the primary branches) prior to treatment, at the end of the growing season (during which treatment(s) were applied), and at the end of the next growing season (during which no treatments were applied). If treatments must be or are allowed to be applied during more than one growing season, then branch angles and all other data in paragraphs (d)(14)(i) and (ii) of this section should be reported at the end of each season treated; and

(B) Effects on vegetative growth as to its appearance (color, size, shape), and growth rates of shoot tips, and trunk. Observations made the growing season following treatment should include effects on winter hardiness, time of leaf expansion in the spring, effects on appearance and growth of vegetative parts, and branch angles.

(ii) The following should also be reported: tree age, variety, rootstock, transplant-to-application interval, stage of vegetative growth at application, other pesticides applied, effects of meteorological conditions on product performance, rates, finished spray volumes applied per unit land area and per tree, extent of spray coverage, number and rates of repeated applications, application-to-observation interval(s), and application equipment.

(iii) Refer to § 94-30 paragraph II.C. for additional guidance.

(15) Products controlling alternate bearing in apples. (i) Comparisons should be made between treated plants and untreated plants with respect to:

(A) Data collected the year of treatment, including: fruitset, fruit drop, yield, delay of maturity, fruit quality (size, shape, color, flavor), and incidence of "bitter pit";

(B) Data collected the year after treatment, including: all factors listed in paragraph (d)(15)(i)(A) of this section, as well as data on the number of blossoms and time of blossoming;

(C) Data collected both in the treated year and the following year, including: performance effects of cultural practices such as pruning and use of plant regulators for fruit thinning and decreasing preharvest fruit drop; effects of environmental conditions such as temperature, frost, and rainfall; and effects on vegetative growth and appearance.

(ii) The following should also be developed: application rates, number of applications, finished spray volume per unit land area and per tree, extent of spray coverage, apple variety, tree age, stage of growth at application, other pesticides applied in the same season, and application equipment.

(iii) If the product is intended for use on the same trees every year, then data in paragraphs (d)(15)(i) and (ii) of this section should be reported for applications repeated more than one growing season.

(iv) Refer to § 94-30 paragraph II.D. for additional guidance.

(16) Products altering sex expression in cucurbits. (i) Comparisons should be made between treated and untreated plants with respect to:

(A) Sex of flowers at each node, number of flowers per plant, vegetative growth rates and appearance, plant stand, time of flowering and harvest, and duration of effectiveness;

(B) Effects of cultural practices and environmental conditions on product performance;

(C) Data in support of hybrid seed production, including number of seeds per fruit, seed yield per acre, percent seed germination, seedling vigor, and seed storage life; and

(D) Data in support of increasing yields in once-over harvest programs, including fruit yield per unit area by grades, market value by grade, and fruit quality (shape, color, and flavor).

(ii) The following should also be reported: application rates, number of applications, stage of growth at application, variety, plant population, application equipment, and finished spray volume per unit area.

(iii) Refer to § 94-30 paragraph II.E. for additional guidance.

(17) Products increasing berry size of seedless table grapes. (i) Comparisons should be made between treated plants (and/or berries) and untreated plants (and/or berries) with respect to:

(A) Berry size, yield (weight per vine), sugar/acid ratio, color, flavor, number of berries per cluster, and effects on vegetative growth (growth rate and formative effects), effect on harvest date, and shelf life;

(B) Effects on flowering in addition to data in paragraph (d)(17)(i)(A) of this section, to be collected and reported for the year following application. (One-half the number of the same plants treated the first growing season must be retreated during the following growing season while the other half will not be retreated); and

(C) Effects of cultural practices, such as girdling, application of other plant regulators, irrigation, and effects of environmental conditions, such as temperature and rainfall, on product performance.

(ii) The following should also be reported: application rates, number of applications, other pesticides applied, stage of growth or maturity at application, application-to-harvest interval, variety, age of planting, finished spray volume applied per unit land area or per vine, extent of spray coverage, and application equipment.

(iii) Refer to § 94-30 paragraph II.H. for additional guidance.

(18) Products decreasing compactness of tightly clustered varieties of grapes. (i) Comparisons should be made between treated plants (and/or berries) and untreated plants (and/or berries) with respect to:

(A) Visual estimate of compactness or looseness, incidence of rot, cluster length, fruit set (number of berries per cluster), number of shot berries per cluster, berry yield (weight per vine), berry quality (size, sugar/acid ratio, color, flavor), effects on vegetative growth (growth rate, formative effects), and effect on rate of crop maturation;

(B) Effects on flowering in addition to data in paragraph (d)(18)(i)(A) of this section, to be collected and reported for the year following application. (One-half the number of the same plants treated the first growing season must be retreated during the following growing season while the remaining half will not be retreated); and

(C) Effects of cultural practices, such as girdling, application of other plant regulators, irrigation, and environmental conditions (such as temperature and rainfall), on product performance.

(ii) The following should also be reported: application rates, number of applications, variety, age of planting, stage of growth or maturation at application, other pesticides applied, finished spray volume per acre or per vine, extent of spray coverage, application-to-harvest intervals, and application equipment.

(iii) Refer to § 94-30 paragraphs II.I. for additional guidance.

(19) Products reducing acidity in citrus. (i) Comparisons should be made between treated plants (and/or commodities) and untreated plants (and/or commodities) with respect to:

(A) Harvest dates when treated and untreated fruit attain their respective harvest maturities. A representative sample of untreated fruit should also be harvested and evaluated, as appropriate for paragraph (d)(19)(i)(B) of this section, at the same time as the treated fruit;

(B) Sugar/acid ratio, fruit yield per tree, juice content, fruit quality (size, shape, color, flavor, blemishes), dollar value, effects on vegetative growth (growth rate and formative effects), effects on development of flowers and fruit present on the tree at application but not intended for treatment, effects on artificially-induced ripening response (coloration), effects on performance of abscission agents, and effects on frost resistance;

(C) Effects on flowering in addition to data in paragraph (d)(19)(i)(A) of this section, to be collected and reported for the year following application. (One-half the number of the same plants treated the first growing season while the remaining half will not be retreated); and

(D) Effects of cultural practices (such as irrigation, pruning, and application of other plant regulators) and effects of environmental conditions (such as temperature and rainfall) on product performance.

(ii) The following should also be reported: application rates, number of applications, stage of plant growth or fruit maturity at application, application-to-harvest interval, crop variety, rootstock, age of planting, other pesticides applied, finished spray volume per unit land area or per tree, extent of spray coverage, application equipment, and fate of fruit (fresh market or processing).

(iii) Refer to § 94-30 paragraph II.J. for additional guidance.

(20) Products inhibiting apple scald. (i) Comparisons should be made between treated fruit and untreated fruit with respect to:

(A) Scald symptoms, number of scalded and unaffected fruit, percent of fruit surface affected, and appearance (severity) of scald-infected areas;

(B) Fruit quality (flesh firmness, color, blemishes, flavor, odor) upon removal from storage, shelf life, and visible residues on fruit surface; and

(C) Effects of varied storage conditions, maturity at harvest, apple varieties, other plant regulators (for ripening, abscission), and tank mixes with other pesticides (such as fungicides) on product performance.

(ii) The following should also be reported: concentration of finished spray or dip, other pesticides applied, extreme weather conditions prior to harvest, method of application, application equipment, storage conditions (duration, temperatures, humidity, and air circulation), harvest-to-application intervals, and duration of exposure (if dipped or fumigated).

(iii) Refer to § 94-30 paragraph II.K. for additional guidance.

§ 94-5 Desiccants.

(a) General. Desiccants may be used for a variety of purposes including: increasing harvest efficiency, reducing plant moisture content, modifying the time of harvest by terminating growth and hastening maturity, or opening up crop foliage to increase canopy penetration by light or air.

(b) Suggested performance standard. Efficacy data in support of desiccant claims need not demonstrate perfect (100%) plant response, but the response attained should be at a level considered commercially acceptable. Commercially acceptable plant response levels vary from one use pattern, plant species or variety, and environmental condition to another; however, the response level must, in all cases, be expected to provide a meaningful benefit to users of the product.

(c) Performance reliability. Efficacy evaluations used for supporting data must be replicated and statistically analyzed where greenhouse or small plot tests are conducted. When commercial application equipment is utilized in large field plots where independently replicated applications may not be practical or economical, efficacy evaluations must be based upon multiple independent observations and/or samples taken from within each treated area. As the response levels approach the limits of usefulness, increased replication and sampling is needed to support efficacy claims and absence of undesired adverse effects.

(d) Specific considerations for plant desiccants. (1) Cotton. (i) Purpose of treatment. The purposes of desiccating cotton plants are to reduce moisture and chlorophyll content, and to desiccate regrowth (after defoliation) where stripper-type harvest equipment is to be used.

(ii) Efficacy data considerations. Data considerations will depend, to some extent, on the intended use of the product. The following considerations are the most commonly appropriate ones for products claiming desiccation of cotton plants:

(A) Label claims for "desiccation" only. Label claims for "desiccation" only should be supported by data which include:

(1) Desiccation evaluation. For both treated plots and untreated checks, the percent dried leaves, stems, and stalks should be determined, using pre- and post-application green versus dried leaf, stem, and stalk counts. Alternatively, visual observations and ratings of the same responses may be appropriate.

(2) Recording intervals. The application-to-maximum effect interval and application-to-harvest interval should be reported.

(3) Percent defoliation. The considerations of § 94-6(d)(1) of this subdivision apply for all cotton defoliation data to be reported.

(4) Effects on terminals. The percentage of cotton plants with dead terminal branches should be reported.

(B) Label claims in addition to "desiccation". When other more specific claims, such as "increased harvester efficiency", are made in addition to "desiccation", the product efficacy should be supported with data pertaining to each specific claim made.

(iii) Concurrent effects. Data from tests should indicate the percent of each stalk killed, extent of regrowth, effects on fiber quality, and effects on premature boll opening. The quantity of regrowth per plant after desiccation (or defoliation) should be determined by harvesting and weighing the regrowth or by visual observations over the period from inception of regrowth until cotton harvest time. The extent of detrimental effects from regrowth should be evaluated and reported in terms of their effects on harvest and quality of the cotton.

(iv) Additional instruction. Refer to § 94-30 paragraph III.A. for additional guidance.

(2) Sorghum. (i) Purpose of treatment. The purposes for desiccating sorghum plants are to decrease moisture in leaves and in immature seedheads arising from tillers, decrease seed germination in heads, and decrease lodging by allowing earlier harvest.

(ii) Efficacy data considerations. (A) Moisture content. The moisture content in seed or other plant parts (depending on purpose of desiccation) should be determined from plants sampled and weighed at treatment and at harvest, and dried in a forced-draft oven until constant dry weight is attained. Percent moisture must be presented as a percent of fresh weight. Desiccation of some plant parts such as leaves may be evaluated visually.

(B) Recording interval. The application-to-maximum effect interval and application-to-harvest interval should be reported.

(iii) Concurrent effects. Effects of the desiccant on the seed, in terms of germination, seedling vigor, color, texture, starch and protein content, flavor, weight, milling properties, and yield should be evaluated in comparison to untreated checks and reported. Untreated check plots should be of sufficient size so that one-half can be harvested at the time of treated plots and the remainder harvested at the usual time for untreated sorghum.

(iv) Additional instruction. Refer to § 94-30 paragraph III.B. for additional guidance.

(3) Irish potato. (i) Purpose of treatment. The purpose for desiccating potato plants is to kill vines prior to harvest. Killing of vines may facilitate both manual and mechanical harvesting; decrease the formation of oversize, misshapen, and second-growth tubers; terminate growth for earlier harvest or to maintain tuber quality; and harden (set) tuber skin.

(ii) Efficacy data considerations. (A) Vine desiccation. The degree of vine desiccation should include evaluation of effects both on leaves and on stems.

(B) Recording intervals. The application-to-maximum effect interval and application-to-harvest interval should be reported.

(C) Secondary claims. Any specific claims other than desiccation, such as maintenance of tuber quality, should be supported with appropriate data.

(iii) Concurrent effects. Effects of the desiccant on tuber yield and quality (grade), storage quality, cooking quality, seed piece quality, flavor, skinning, vascular discoloration, and sprouting should be evaluated in comparison to untreated checks and reported.

(iv) Additional instruction. Refer to § 94-30 paragraph III.C. for additional guidance.

(4) Sunflower. (i) Purpose of treatment. The purposes of desiccating sunflower plants are to reduce the quantity of green (fresh) plant material passing through mechanical harvesters and to reduce moisture in the seed heads.

(ii) Efficacy data considerations. (A) Harvester efficiency. Reduction in bulk is indicated in increased harvester efficiency. Harvester efficiency should be expressed in terms of length of time required for harvesting treated and untreated plots of equal size and stand.

(B) Moisture content. Moisture content of seeds may be determined in a manner similar to that for sorghum. See paragraph (d)(2)(ii)(A) of this section.

(C) Recording intervals. The application-to-maximum effect interval and application-to-harvest interval should be reported.

(iii) Concurrent effects. Effects of the desiccant on seed yields, flavor, and quality should be evaluated by comparison with untreated checks and reported.

(iv) Additional instruction. Refer to § 94-30 paragraph III.D. for additional guidance.

(5) Seed crops. (i) Purpose of treatment. The purposes of applications of desiccants to crops such as alfalfa, clover, dry beans, and other crops grown for seed production are to reduce moisture content and increase harvester efficiency.

(ii) Efficacy data considerations. The efficacy data considerations for seed crops are similar to those for sunflowers. See paragraph (d)(4)(ii) of this section.

(iii) Concurrent effects. Effects of the desiccant on seed yields, pod dehiscence, seed weight, seedling vigor, and percent germination should be evaluated by comparison to untreated controls and reported.

(6) Onion. (i) Purpose of treatment. The purposes for desiccating onion plants are to dry tops, facilitate harvest, and allow flexibility in scheduling of harvest.

(ii) Efficacy data considerations. (A) Moisture content. Moisture content of tops should be reported and may be determined in a manner similar to that for sorghum [see paragraph (d)(2)(ii)(A) of this section] or may be evaluated visually.

(B) Harvest efficiency. Increased harvest efficiency, if claimed, should be expressed in terms of length of time required for harvesting treated and untreated plots of equal size and stand.

(C) Recording intervals. The application-to-maximum effect interval and application-to-harvest interval should be reported.

(iii) Concurrent effects. Effects of the desiccant on onion yields, bulb weight, sunburn of bulbs, and flavor should be evaluated by comparison to untreated checks, and reported.

(iv) Additional instruction. Refer to § 94-30 paragraph III.E. for additional guidance.

(7) Sugarcane. (i) Purpose of treatment. The purpose for desiccating sugarcane plants is to dry leaves for improved burning which may increase harvest and refining efficiency.

(ii) Efficacy data considerations. (A) Moisture content. Leaf desiccation should be evaluated by comparison to untreated check plots and reported. Leaf desiccation may be determined by visual observation of browning or by determining the percent moisture by drying leaves (minus the midribs) to a constant dry weight in a forced-draft oven.

(B) Burning efficiency. If improved burning is claimed, visual estimates of percent foliage burned off compared to burned untreated check plots, or weight of trash remaining on stalks after burning both treated and untreated check plants, should be reported.

(C) Recording intervals. The application-to-maximum effect interval and application-to-harvest interval should be reported.

(iii) Concurrent effects. Effects of desiccant application on sugarcane sucrose yield per unit area, ratoon growth, and effects on growth of the terminal buds of seed pieces should be evaluated by comparison to untreated check plants, and reported.

(iv) Additional instruction. Refer to § 94-30 paragraph III.F. for additional guidance.

§ 94-6 Defoliants.

(a) General. Defoliants may be used for a variety of purposes, many of which are the same as indiated for desiccants. Since the purpose of defoliation is the removal of plant leaves (or, occasionally, other aboveground parts), the percent leaf drop should be determined by making pre- and post-application leaf counts or by visual observation, for both treated and untreated plots.

(b) Suggested performance standard. Efficacy data in support of defoliant claims need not demonstrate perfect (100%) plant response, but the response attained must be at a level considered commercially acceptable. Commercially acceptable plant response levels vary from one use pattern, plant species or variety, and environmental condition to another; however, the response level should, in all cases, by expected to provide a meaningful benefit to the user of the product.

(c) Performance reliability. Efficacy evaluations should be replicated and statistically analyzed where greenhouse or small plot tests are conducted. When commercial application equipment is utilized in large field plots where independently replicated applications may not be practical or economical, efficacy evaluations should be based upon multiple independent observations and/or samples taken from within each treated area. As the response levels approach the limits of usefulness, increased replication and sampling is necessary to support efficacy claims and absence of undesired adverse effects.

(d) Specific considerations for defoliants. (1) Cotton. (i) Purpose of treatment. The primary purpose of defoliant application to cotton is leaf removal which in turn may facilitate harvest by reducing trash and improving harvester efficiency, may preserve seed and fiber quality, or may cause mature green bolls to open sooner.

(ii) Efficacy data considerations. (A) Defoliation evaluation. The percentage of leaf drop must be supported by comparison with untreated controls, and reported.

(B) Harvest efficiency. If claimed, increased harvest efficiency should be supported by comparison of length of time required to harvest treated and untreated plots of equal size and stand. Harvest efficiency may include comparisons of the amount of unpicked lint residues remaining after harvest of treated and untreated plots.

(C) Secondary claims. If other secondary effects of defoliation are claimed in labeling, specific data considerations will be applied, as appropriate, on a case-by-case basis.

(D) Recording intervals. The application-to-maximum effect interval and the application-to-harvest interval should be reported.

(iii) Concurrent effects. Effects of defoliant applications on cotton yields, boll abscission, immature boll opening, regrowth and cotton quality (lint spotting and staining; fiber length, strength, and diameter; seed oil content; seed germination; seed vigor; and trash content) should be evaluated by comparison to untreated check plots, and reported.

(iv) Additional instruction. Refer to the § 94-30 paragraph IV.A. for additional guidance.

(2) Okra. (i) Purpose of treatment. Defoliants are applied to okra to remove leaves and facilitate harvest.

(ii) Efficacy data considerations. (A) Defoliation evaluation. The percentage of leaf drop should be evaluated by comparison with untreated controls, and reported.

(B) Harvest efficiency. If claimed, increased harvest efficency should be supported by comparison with the length of time required to harvest treated and untreated plots of equal size and stand.

(C) Recording intervals. The application-to-maximum effect interval and applicator-to-harvest interval should be reported.

(iii) Concurrent effects. Effects of the defoliant on okra yield, flavor, and pod damage should be evaluated by comparison to untreated check plants, and reported.

(3) Deciduous hardwoods and ornamentals in nurseries. (i) Purpose of treatment. Application of defoliants to these plants is for leaf removal prior to storage, harvest, or shipping.

(ii) Efficacy data considerations. (A) Defoliation evaluation. The percentage of leaf drop should be evaluated by comparison with untreated controls, and reported. If partial defoliation occurs, data should be provided illustrating any differentiation between the extent of defoliation on new and old wood.

(B) Secondary claims. If secondary effects, such as increased survival, are claimed in labeling, then appropriate supporting data, such as percent survival, are needed.

(C) Recording intervals. The application-to-maximum effects interval and application-to-harvest (storage or shipping) interval should be reported.

(ii) Concurrent effects. The following should be evaluated and reported: effects of the defoliant on bark (desiccation, discoloration), on stem growing points (tip dieback) and growth rates, and on dormant or expanding buds by comparison to untreated check plants. Evaluations should be made just prior to application, at the time of storage, harvest, or shipping, and at the flush of spring growth following application.

(iv) Additional instruction. Refer to § 94-30 paragraph IV.B. for additional guidance.

§ 94-30 Acceptable methods.

In general, several test methods are necessary to prove the usefulness of a given pesticide product. The methods selected should test the product in the manner in which it is to be used and in a manner which will show the effect of field variables on the product's usefulness. The data obtained should support the product label claims and must be representative of the results that the grower will obtain from commercial usage of the product. While data considerations under specific situations may be identified, the validity of test methods used to generate the data will be evaluated on a case-by-case basis in the scientific review process. Due to the number, variety, specificity, and continuing evolution of valid test methods for performance evaluation of terrestrial herbicides, plant regulators, desiccants, and defoliants, the following test methods and references are cited only as examples of test methods which may generate a portion of the product performance data for registration purposes.

I. Terrestrial herbicides.

Methods reported in the literature for testing the usefulness of herbicides vary with the physiological and biochemical characteristics of the herbicide, the formulation, crop, geographical location, application equipment, cultural practices, and other factors. Instead of citing a multitude of very specific test methods having very limited usefulness, the Agency has cited two published works which reflect several basic methods common to many herbicide evaluations.

A. Herbicide application. Wiese (1977) discusses the importance of precise and uniform application of herbicides, along with the equipment, techniques, and procedures required to obtain such application. One prime consideration in the application of herbicides, whether in small plot or large-scale trials, is uniformity of compound distribution and coverage.

B. Measuring plant responses. Frans and Talbert (1977) discuss plot designs and field evaluation procedures along with statistical treatment. For registration purposes, adequate counts and/or ratings must be taken to show the effectiveness of the compound against individual weed species and to show crop safety. The crop injury and weed control evaluations must be interpreted in terms of "acceptability". This can be done for weed control by reference to phytotoxicity, yield, and quality (grades) of the commodities from those check plots where acceptable levels of weed control are maintained.

References.

Frans, R.E., and R.E. Talbert. 1977. Design of Field Experiments and the Measurement and Analysis of Plant Responses. Pp. 15-24 in Research Methods in Weed Science. B. Truelove, ed. 2nd Ed. S. Weed Sci. Soc. Auburn Printing, Inc.: Auburn, Ala. 221 pp.

Wiese, A.F. 1977. Herbicide Application. Pp. 1-14 in Research Methods in Weed Science. B. Truelove, ed. 2nd Ed. S. Weed Sci. Soc. Auburn Printing, Inc.: Auburn, Ala. 221 pp.

II. Plant regulators.

Many bioassay, laboratory, and greenhouse methods have been published for the detection of chemicals which modify plant growth, development, and response. Only a few published methods are available, however, for evaluating the potential commercial utility of these chemicals under field conditions.

Testing under actual field conditions remains the only acceptable method for evaluating the efficacy of potential plant regulators. Because responses obtained with plant regulators cover such a wide range (for example, bud inhibition, berry size increase, and fruit coloring), specific field test methods are outlined. These are based to a large extent on methods employed to evaluate the efficacy of plant regulators presently marketed. New methods and protocols for evaluating plant regulators are continually being developed because both new uses and new chemicals are being sought. In all cases, however, data obtained from field tests should support the label claims for the product and must be representative of the results that the producer can expect when the product is used in accordance with label instructions under commercial conditions.

A. Control of axillary bud (sucker) growth on tobacco. Plant regulating chemicals have been developed which inhibit the growth of axillary suckers. These growth-inhibiting chemicals may be divided into two types: systemic and contact. The systemic regulators are applied as sprays to leaves, are absorbed by the plant, and are translocated throughout the plant, but especially to the meristematic areas of the axillary buds. The contact-type sucker control chemicals are also applied as sprays but are directed down the stalk so that they contact the axillary buds. They kill the small suckers without damaging more mature leaf tissue. Test methods and factors affecting the efficacy of various tobacco sucker control agents have been discussed in a number of publications (Collins et al., 1972; Seltmann and Priest, 1972; Seltmann and Marshall, 1967; Smith et al., 1971; Steffens et al., 1969a; Steffens et al., 1969b; and Spaulding et al., 1970).

1. Scope.

1.1 The Regional Tobacco Growth Regulator Committee, composed of State and Federal researchers, extension workers, representatives of tobacco and chemical industries, and representatives of the Environmental Protection Agency, have worked together to evaluate new plant regulating chemicals potentially useful in tobacco production. The following tests and procedures describe those used by the State and USDA personnel on the Committee in evaluation of new plant regulators for tobacco. As such, the following wording, procedures, and guidance are those of the Committee and not necessarily those of EPA. While EPA is not bound by Committee requirements or decisions, Committee personnel are the recognized experts in tobacco plant regulators, and prospective registrants are encouraged by EPA to participate in the Committee's program. EPA cannot specifically require comparisons with "standard products" or public revelation of active ingredients as indicated in Committee requirements; however, the detailed types of data required by the Committee do correspond to EPA specifications for efficacy, phytotoxicity, yield, and chemical and physical properties, quality, and flavor of cured leaves. Any such data generated for the Committee will also, upon submission to EPA, be considered as partial support for product registration. Consequently, the Committee's procedures and requirements serve well in providing meaningful guidance toward product registration. Two types of field tests are conducted annually, usually at 15 locations in 10 states. Tobacco is evaluated with respect to efficacy of treatment, agronomic characteristics, commercial utility, chemical and physical properties, and smoke taste. Leaf samples of known history are made available for assessment of residues of residues of applied chemicals.

1.2 The flow chart (Figure 1) summarizes the progression of tests established. After each test, the Committee will evaluate the data to determine if any entry is eligible for the next test in the progression. After company proprietary tests, the Committee makes recommendations to the Environmental Protection Agency regarding label registration.

2. Test chemicals.

2.1 New chemicals: These include structures, mixtures, sequential treatments of chemicals, or management-chemical regimes not previously tested. Two or more chemicals to be treated in sequential applications or as management-chemical regimes will be considered "one" treatment. If a management-chemical regime involves a new chemical or a new management procedure, it will be considered a new chemical treatment.

2.2 Modified chemicals: These include formulations of previously approved chemicals which have been slightly altered (salt form, surfactant, etc.). The company submitting the chemical must supply data to indicate if the entry is to be considered a modified chemical.

3. Types of tests and test methods.

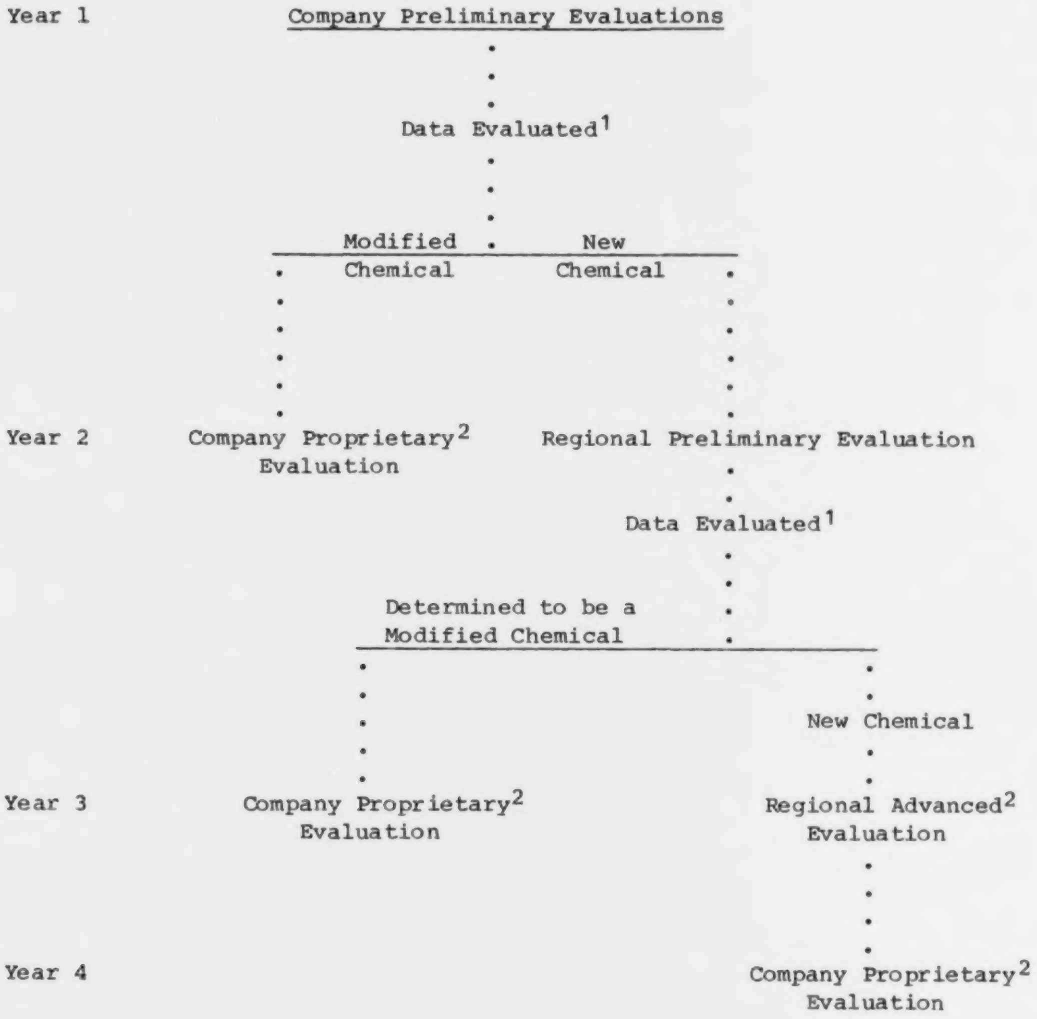
There are 4 sequential levels of testing: company preliminary tests: regional preliminary tests; regional advanced tests; and company proprietary tests.

3.1 Company preliminary tests. These tests are field trials conducted by chemical companies to provide data to justify entry of a chemical into the regional preliminary testing program.

3.1.a. Test procedures.

3.1.a.1. Minimum of 2 flue-cured and 2 burley tobacco locations, if the chemical is to be entered in both flue-cured and air-cured tobacco classes. Both locations for any one class are to be in the area where the class is commercially produced and in different environmental areas.

Figure 1. Sequence of evaluation for tobacco sucker control products.


Year 1
Company Preliminary Evaluations
Data Evaluated[1]
Modified Chemical
New Chemical
Year 2
Company Proprietary[2] Evaluation
Regional Preliminary Evaluation
Data Evaluated[1]
Determined to be a Modified Chemical
New Chemical
Year 3
Company Proprietary[2] Evaluation
Regional Advanced[2] Evaluation
Year 4
Company Proprietary[2] Evaluation

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[1] Evaluation by Committee to determine test level required.

[2] After Regional Advanced and Company Proprietary Evaluations, the Committee makes recommendations to the Environmental Protection Agency regarding label registration. Experimental use permits are optional.

3.1.a.2. Non-replicated fifteen-plant plots minimum.

3.1.a.3. Efficacy based on sucker weight; the treatment should be 90 percent as effective as the performance of the appropriate standards included in the same test. Data are to be obtained for flue-cured tobacco 2 weeks after treatments (observational) and at final harvest (sucker number and green weight). For burley tobacco, sucker number and green weight are to be taken at harvest.

3.1.a.4. Leaf phytotoxicity data (including necrosis, malformation, etc.) taken at the appropriate stage (scale 0 to 5); leaf drop and stalk girdling are not to exceed that of appropriate standards.

3.1.a.5. Records of cultural practices and environmental conditions.

3.2. Regional preliminary tests: These tests are conducted by State or Federal agricultural experiment station scientists who are Committee members.

3.2.a. General testing procedures.

3.2.a.1. The formulation of the chemical (or very closely related formulation) must have been evaluated on field-grown tobacco.

3.2.a.2. Each person conducting the test must know the identity of the active ingredient on a confidential basis. The overall committee coordinator may request the chemical composition of the complete formulation on a confidential basis.

3.2.a.3. The company is to supply all information available on toxicity to humans, animals, etc., and any information available on the breakdown, persistence, action, etc., of the chemical.

3.2.a.4. The company is to supply sufficient quantities of the formulated chemical to conduct the evaluations at all proposed locations. The formulated chemical appropriately packaged is to be shipped by the company to the individual locations by the specific dates as per instructions supplied by the Committee.

3.2.a.5. The company if submitting the chemical is to indicate if the chemical is new or modified and suggest in writing the rate of application, the procedure for application, and the timing for application. The company is also to suggest the procedure for harvesting and the sequence for curing if different from the standard field practice.

3.2.a.6. After treatment, and during the course of the field evaluation, the research worker will determine by visual observation the potential of the treatment for achieving the desired results. This information will be immediately forwarded to the coordinator for that tobacco class so information on efficacy across a particular class can be summarized. After a decision is made by the coordinator, based on the information available, complete data will be collected from only those treatments showing the desired results.

3.2.b. Field test procedures and data procedures.

3.2.b.1. Suggested distribution for flue-cured tobacco, 6 locations; for burly tobacco, 4 locations; for cigar filler tobacco, 2 locations; for dark tobacco, 2 locations; and for Maryland tobacco, 1 location.

3.2.b.2. A minimum of thirty-plant plots (non-replicated).

3.2.b.3. Agronomic observations, including:

-- Efficacy based on percent sucker control by weight, number of suckers per plant, and weight per sucker;

-- Leaf phytotoxicity (including necrosis, malformation, etc.) data taken at a maximum manifestation (0 to 5 scale). Leaf drop and stalk-girdling information is to be reported; and

-- Yield and quality based on U.S. standard grades.

3.2.b.4. Cured leaf data (on successful entries only), including:

-- Percent useable by treatments and locations. Evaluations conducted by tobacco companies;

-- Chemical and physical properties (composite sample by treatment for each class)(see Table 1); and

-- Evaluation of smoke taste for the identification of unacceptable off-taste due to treatment. Tobacco companies conducting smoke panel tests must know the identity of applied chemicals. Cigarettes will be made from a composite sample by treatment for each class (flue-cured and burley).

3.2.c. Procedures for passing the regional preliminary test.

3.2.c.1. Minimum sucker control based on sucker weight must be 90% as effective as the performance of the appropriate standards included in the same test.

3.2.c.2. Leaf phytotoxicity -- (including necrosis, malformation, etc.). Average of 2.5 or less (0-5 scale). Leaf drop and stalk girdling are not to exceed appropriate standards.

3.2.c.3. Yield, quality based on U.S. standard grades, tobacco company evaluations, physical and chemical properties, and smoke taste tests must compare favorably with appropriate standards.

3.3. Regional advanced tests. After successfully passing the preliminary tests, chemicals may be entered in advanced tests, also conducted by State and Federal scientists-Committee members.

Table 1. Chemical and Physical Property Analyses

Flue-Cured Tobacco	Burley Tobacco
Nicotine or total alkaloids	Nicotine or total alkaloids
Reducing sugars	Total nitrogen
Total volatile bases minus nicotine	Total volatile bases minus nicotine
Total ash	Total ash
Equilibrium moisture content	Equilibrium moisture content
Filling value	Filling value
	Alkalinity number of water-soluble ash

3.3.a. General considerations.

3.3.a.1. To enter the Regional Advanced Evaluations, an entry should have passed Regional Preliminary Evaluations.

3.3.a.2. The identity of the active ingredient of an entry should be revealed as public information. The overall coordinator may request the chemical composition of the complete formulation on a confidential basis.

3.3.a.3. The company is to supply all information available on toxicity to humans, animals, etc. Any information available on the breakdown, persistence, action, etc., of the chemical should also be supplied.

3.3.a.4. The company is to supply sufficient quantities of the formulated chemical to conduct the evaluation at all locations proposed. The formulated chemical appropriately packaged is to be shipped by the company to the individual locations by specific dates supplied by the Committee.

3.3.b. Field test and data procedures.

3.3.b.1. Suggested distribution for flue-cured tobacco, 6 locations; for burley tobacco, 4 locations; for cigar filler tobacco, 2 locations; for dark tobacco, 2 locations; and for Maryland tobacco, 1 location.

3.3.b.2. Plot size -- randomized block design with 4 replicates, 30 plants/replicate.

3.3.b.3. Agronomic data, including:

-- Efficacy based on percent sucker control by weight, sucker number per plant, and weight per sucker;

-- Leaf phytotoxicity data (including necrosis, malformation, etc.) taken at maximum manifestation (0 to 5 scale). Leaf drop and stalk girdling information is to be reported; and

-- Yield and quality based on U.S. standard grades.

3.3.b.4. Cured leaf data, including:

-- Usability, body, and texture for flue-cured, and usability and quality rating for burley (by location) is to be evaluated by tobacco companies;

-- Chemical and physical property determinations by locations (see Table 1); and

-- Evaluation of smoke taste for the identification of unacceptable off-taste due to treatment. Tobacco companies conducting smoke panels should know the identity of the applied chemicals. Cigarettes will be made from a composite sample by treatment for each class (flue-cured and burley).

3.3.c. Procedures for passing the regional advanced test.

3.3.c.1. Sucker control -- based on sucker weight, control must be 90% as effective as the performance of the appropriate standards included in the evaluation.

3.3.c.2. Leaf phytotoxicity -- (including necrosis, malformation, etc.). Average of 2.5 or less (0 to 5 scale). Leaf drop and stalk girdling are not to exceed appropriate standards.

3.3.c.3. Yield and quality based on U.S. standard grades must compare favorably with appropriate standards.

3.3.c.4. Tobacco company evaluations -- The cured leaf from treated plants must compare favorably with appropriate standards.

3.3.c.5. Chemical and physical properties -- When the data are statistically analyzed, the 0.5% level of significance will be used to determine if the specified chemical and physical properties compare favorably with appropriate standards.

-- Flue-cured -- The specified chemical and physical properties should be within the range of the standard hand-suckered check and the standard sequential treatment, or be better than the hand-suckered check.

-- Burley -- The specified chemical and physical properties should be within the range of the standard hand-suckered check and the standard chemical treatment, or be better than the hand-suckered check.

3.3.c.6. Smoke taste -- These tests are designed to ascertain if the chemical treatment imparts an off-taste to the smoke of cigarettes manufactured from treated tobacco. They are to be conducted as comparison tests using a known check to compare a treated sample. A coded check sample will be used as an internal standard. In addition to the simple evaluation of "acceptable" or "not acceptable," the percentage and the number of panelists will also be reported by each tobacco company making smoke panel tests. Each company is to specify which treatment, if any, is considered objectionable. If a majority of the companies reject the treatment, after the internal standard is considered, the treatment will be dropped from further evaluations.

3.4. Company proprietary tests. The chemical company sponsoring a chemical is responsible for conducting these final "on-farm"-type tests. All data is to be submitted to the Regional Committee for evaluation.

3.4.a. General considersations.

3.4.a.1. Using the same formulation evaluated in the advanced and/or preliminary tests, the company is to conduct field tests at minimum of 3 flue-cured and/or 2 burley tobacco locations.

3.4.a.2. Applications should be made with mechanical sprayers similar to those used in the commercial production of tobacco.

3.4.b. Field test and data procedures.

3.4.b.1. Distribution of field locations (minimum number). For flue-cured tobacco, 1 southern (Fla., Ga., or S.C.); 1 eastern (N.C.); and 1 old belt (N.C., Va.). For burley tobacco, 1 high elevation (N.C., Va.) and 1 lower elevation (Tenn., Ky.).

3.4.b.2. Suggested plot size -- 1/10 acre when as single or 1/40th acre small plots replicated three times. Locations within the same general area can be considered as replicates. Data are to be taken on at least three 10-plant subsamples. One 30-plant topped-but-notsuckered plot is to be included for calculation of percentage sucker control.

3.4.b.3. Agronomic data, including:

-- Efficacy based on percent sucker control by weight, sucker number per plant, and weight per sucker; and

-- Phytotoxicity at maximum manifestation (0 to 5 scale). Leaf drop and stalk girdling information is to be reported.

3.4.c. Modified chemicals and/or formulations. Modified chemicals and/or formulations going through Company Proprietary Evaluations which have not been subjected to smoke tests will require such tests in comparison to appropriate standards. Approximately 25 lbs. of cured tobacco are required from each treatment for making cigarettes used for smoke taste evaluations.

3.4.d. Evaluation standards.

3.4.d.1. Treatments (applied in accordance with label instruction):

-- Contact -- fatty alcohol (FA) normal primary octyl-decyl or decyl alcohols.

-- Systemic -- diethanolamine salt of maleic hydrazide (MH).

-- Sequential -- fatty alcohol followed by maleic hydrazide.

-- Untreated -- Handsuckered (HS) (suckers are to be removed when they are 4 to 6 in. long) and topped-and-not-suckered (TNS).

3.4.d.2. Treatments for each tobacco type:

-- Flue-cured -- The standard treatments should include the TNS, HS, and the sequential treatments except that the HS is not required in Company Preliminary and Company Proprietary Evaluations.

-- Burley -- The standard treatments should include the TNS, HS, and the MH treatments expect that the HS treatment is not required in Company Preliminary and Company Proprietary Evaluations.

3.4.d.3. The degree of sucker control is measured as percent by which treatment reduces sucker green weight compared with TNS check - 90% as effective as appropriate standards included in the evaluations.

3.4.d.4. Leaf phytotoxicity is rated either as:

-- Leaf injury -- on a 0 to 5 scale (0 = none; 1 = very slight; 2 = slight; 3 = slight to moderate; 4 = moderate; and 5 = severe).

Rating to include necrosis, malformation, etc.; or

-- No leaf drop or stalk girdling compared to appropriate standards.

3.4.d.5. Tobacco company evaluations (visual):

-- The data should include percent usable cured tobacco by the evaluating tobacco companies (average and total usable tobacco by at least one company).

-- Regional Preliminary -- report percent usable only.

-- Regional Advanced -- report flue-cured, percent usable, and leaf body and texture evaluations; burley, percent usable and quality rating.

-- The cured leaf from treated plants must compare favorably with the standard HS and sequential treatments for the flue-cured and with the standard HS and the chemical treatment for the burley tobacco class.

3.4.d.6. Chemical and physical properties:

-- When the data are statistically analyzed, the 0.05 level of significance will be used to determine if the specified chemical and physical properties compare favorably with the standards.

-- Flue-cured -- The specified chemical and physical properties should be within the range of the standard HS check and the standard sequential treatment, or be better than the HS check.

-- Burley -- The specified chemical and physical properties should be within the range of the standard HS check and the standard chemical treatment, or be better than the HS check.

References.

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B. Control of tobacco leaf ripening.

1. Scope.

1.1. Chemical ripening agents which hasten leaf yellowing may allow for the more efficient utilization of harvesting and curing equipment by enabling enabling the grower to harvest and cure tobacco when the equipment normally would be idle. The producer may also be better able to program the harvesting and curing of crops which tend to mature faster or slower than normal due to weather conditions. It has been shown that tobacco leaves chemically ripened in the field undergo chemical changes and loss of moisture which normally occur in the yellowing stage of curing. Because of these changes, the length of the curing cycle can be reduced by one or two days which results in increased barn availability and a slight reduction in usage of curing fuel and electricity in bulk barns.

1.2. Time and care are also involved in curing and handling the air-cured tobacco classes. Chemical treatments which cause some loss of moisture prior to harvest would allow more efficient utilization of barns because the tobacco could be spaced closer together. Chemical treatments which shorten the curing time would also reduce the risk of barn rots and other similar problems.

1.3. In an effort to hasten yellowing of tobacco that remained in an immature state, plants have been treated with herbicides (Seltmann and Peedin, 1968). It has long been known that ethylene gas can have dramatic physiological effects on plants. Gases and mixtures of gases that include ethylene have been introduced into curing barns in order to hasten the uniform development of desired leaf color. The effects on plants of chemicals which act through an ethylene evolution system have been reported (deWilde, 1971). Such chemicals have been evaluated on tobacco and have been found to have potential for yellowing leaves under field conditions and thus have practical implications (Cutler and Gaines, 1971; Miles et al., 1972; Steffens et al., 1970). The concept of controlled yellowing or ripening of tobacco has gained considerable interest, and candidate chemicals as well as management-chemical regimes are being actively sought and evaluated.

1.4. As in the case of tobacco sucker control agents, the Regional Tobacco Growth Regulator Committee has generated a set of guidelines for tobacco ripening agents which parallels the sequential evaluation scheme for such control agents. The previous section (II.A.) of this Appendix must be read, since, with the exception of efficacy-related data, data considerations addressing tobacco classes, test locations and sequence, plot sizes, phytotoxicity, yield and quality, and cured leaf data (percent usable, chemical and physical properties, and smoke taste) are identical.

2. Data considerations are as follows:

2.1. Company preliminary evaluations. Data are to be obtained on the exhibition of the "ripening" response, speed of ripening, and color development or reduction in curing time in the barn compared to appropriate standards.

2.2. Regional preliminary evaluations.

2.2.a. Efficacy data -- Record degree of ripening in relation to standards by estimating percentage of yellowing and uniformity of color development with respect to time. Record length of curing time compared to standards.

2.2.b. Requirements for passage of regional preliminary evaluations -- include the obvious increase in the rate of yellowing and/or curing.

2.3. Regional advanced evaluations.

2.3.a. Efficacy data -- Record degree of ripening in relation to standards by estimating percent of yellowing and uniformity of color development with respect to time. Record length of curing time.

2.3.b. Requirements for passage of regional advanced evaluations:

2.3.b.1. Efficacy -- Obvious increase in the rate of yellowing and/or curing.

2.3.b.2. Tobacco company evaluatons -- The cured leaf from treated plants must compare favorably with the appropriate standards.

2.3.b.3. Chemical and physical properties -- Specified chemical and physical properties should compare favorably with those of the standard.

2.4. Company proprietary evaluations. Efficacy data recorded should include the ripening of the treated leaf, the rate of color development, and the uniformity of ripening of the average leaf. The effect on curing time in the barn should be recorded.

2.5. Evaluation standards.

2.5.a. Ripening:

2.5.a.1. Flue-cured -- Conventional harvesting and curing procedures are to serve as the standard for chemical ripening agents. An ethephon treatment should be included as a basis for comparison but is not to serve as the standard.

2.5.a.2. Burley and other air-cured classes -- Conventional harvesting and curing procedures will be the standard.

2.5.a.3. The method for the control of sucker growth on tobacco evaluated in ripening experiments should be uniform over all treatments in the experiment.

2.5.b. Degree of ripening: The treatment should show an obvious improvement in rate of yellowing and/or curing in comparison to conventional practices, and the cured leaf from treated plants must compare favorably with the appropriate standards.

2.5.c. Tobacco company evaluations (visual): The cured leaf from treated plants must compare favorably with conventional harvesting and curing practices.

2.5.d. Chemical and physical properties: The specified chemical and physical properties should compare favorably with conventional harvesting and curing procedures.

References.

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C. Control of white (Irish) potato tuber sprouting.

1. Types of plant regulator applications to induce dormancy.

In recent years, several types of plant regulator applications have been used to successfully induce dormancy:

(a) Applying the product as preharvest applications;

(b) Dipping tubers in a solution of the product before storage;

(c) Dusting the tubers with the product before storage;

(d) Applying the regulating chemical as a volatile suspendable solid to the tubers while in storage; or

(e) Applying the product as a spray or wax solution to the tubers as they are packaged for the retail market. Factors (such as storage temperature) affecting potato sprout inhibition and methods of testing have been discussed in several publications (Sparks and Summers, 1974; Zaehringer et al., 1966; Rao and Wittwer, 1955; Hruschka et al., 1965; Nylund and Ayres, 1964; Cunningham et al., 1966; and Weaver, 1972).

2. Test conditions. The usual procedure is to randomly collect about 400 tubers for each variable to be tested. They should be field-run quality but with no green, rot, or serious malformations. These are divided into 4 replicates of 100 tubers each. The method of applying the inhibitor depends upon the time at which the material is to be applied. Several dosage levels of the chemical under test are necessary.

In the case of preharvest applications of plant regulators to potato plants, variables (such as time of application, dosage of chemical applied, time of harvest, and effect of rainfall) must be considered in addition to subsequent storage conditions. Replicated plots (usually 4 replicates) sufficiently large to allow statistical analysis should be used.

Treated tubers or tubers from treated plants should be stored at various temperatures, subjected to various airflows and treated with air of different humidity levels to determine length of control (Sparks, 1965 and Sparks, 1973). Retreatment with the inhibitor may be required if the material is volatile and high rates of airflow are used. For comparison purposes, untreated tubers and tubers treated with standard sprout inhibitors are to be included. Standard chemicals may include CIPC (isopropyl-*m*-chlorocarbanilate), TONE (1,2,4,5-tetrachloro-3-nitrobenzene), maleic hydrazide, or other chemicals registered for this use.

3. Interpretation of test results.

a. At monthly intervals for 12 mo., data on weight loss, rot, and sprouting should be taken.

b. At the end of the storage period, other data are also usually obtained:

i. Weight loss.

ii. Rot loss.

iii. Sprouting.

iv. Flattening and shriveling.

v. Effect on suberin and wound periderm formation.

vi. Heat generated in the potato pile (a gross measure of respiration rate.

vii. Quality change (sugars, mealiness, fry color, flavor, chipping).

viii. Appearance change (loss of net or russetting).

ix. Utilization as propagation stock.

References.

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D. Control of branch angle in young trees. In many trees, especially apples and pears, the primary branches form narrow angles with the main trunk which result in structurally weak crotches that often break under moderate or heavy fruiting and seriously reduce the productiveness of the tree. Also, such narrow crotches are very prone to winter injury. Within a few years the entire tree may be lost from further weakening and secondary infection. The development of wide branch angles avoids the bark inclusion in the tree crotches and trees can bear heavier crops when they begin fruiting. The more open growth also permits more light into the center of of the tree, which is particularly useful on spur type

Delicious apples or regular Delicious on dwarfing stock. Factors affecting chemical treatments to modify crotch angle and methods of testing to demonstrate the efficacy of a chemical treatment have been discussed in several publications (Verner, 1938; Bukovac, 1963, 1968).

1. Test conditions. Young fruit trees, usually in the second or third year after being set in the field, are sprayed with several concentrations of the test chemical. The correct timing of application must be determined [usually when the new shoot growth is 3 to 5 in. (7.6 to 12.7 cm) long]. The angle at which the branch leaves the main stem should be measured at the end of the growing season. The effect on vegetative growth also should be measured.

a. Some factors affecting product performance:

i. Volume of spray may be important. The trees should be sprayed to runoff until sufficient data show that lower volumes can be used.

ii. Since response to chemical treatment can vary widely with variety, the effect on all varieties should be studied.

iii. Tests should be made for each variety to determine the rate [lb. active ingredient/100 gal. (kg/1) of spray solution and volume of spray solution applied per tree] and the stage of development of the tree at the time of application that will give the best response under the environmental conditions where the product will be used.

iv. The effect of seedling vigor and rate of growth should be determined, especially in relation to inhibition of vegetative growth.

v. The effect of rainfall following application should be determined.

vi. The effect of a repeat application the following year should be determined.

b. Interpretation of test results. To support label claims for modifying crotch angle development in fruit trees, the treatment should increase the angle at which the branch leaves the main stem. This is more important than just causing the outer part of the branch to bend downward. In general, the angle should be greater than 45° to insure wide angle laterals for a sounder structural framework in trees.

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Verner, L. 1938. The effect of a plant growth substance on crotch angles in young apple trees. Proc. Am. Soc. Hort. Sci. 36:415-422.

E. Control of alternate bearing in apples. Many varieties of apples bear heavily one year ("on" year) and very little the following year ("off" year). Chemical treatment (return bloom treatment) applied in the "on" year before blossom differentiation can increase yield during the "off" year. In addition to leveling out crop production for the 2-year cycle, such treatments often result in an increase in total yield for the period.

1. Test conditions. A series of test trees, in the "on" year, should be paired on the basis of uniformity of rootstock, tree size, and the intensity of blossoming in the spring of the year. One tree of each pair is sprayed with the chemical treatment; the other should remain untreated. Each treatment should be sprayed to runoff until data are accumulated to show that lower volumes are equally effective. Various rates need to be tested on each variety to determine the optimum rate. Timing of application must also be determined. (The optimum timing usually is 4 to 5 weeks after full bloom.) To maintain uniform crop production, it is necessary to treat only during the "on" year. Because the degree of blossoming in the spring will determine those years in which treatments should be made, studies are needed on each variety on the degree of spring blossoming and the amount of return bloom the following season.

Since climatic conditions can affect performance, it is absolutely necessary to test the compound in each location where it will be used. Growers should be cautioned to start with a small block of trees until they gain experience in the performance of the compound under their environmental conditions. Any effect of rainfall following application should be determined.

The effect of a blossom-thinning treatment or the occurrence of frost on blossom set should be determined before the application of the return bloom treatment. Studies with various degrees of blossom thinning are needed to determine the rate and effect of the return bloom treatment.

Tree vigor and size, as well as the mineral nutrition of the tree, also can affect response.

2. Interpretation of test results. The effects of any treatment on the control of biennial bearing will be noted in the year following treatment. The yield should be taken at the end of the second season and compared with untreated trees. However, since the objective is to even out the productivity of the tree, yields should also be taken at the end of the first season and the total yield for the 2 years should be compared.

Normally, yield alone will not give the total picture, so it is necessary to grade the fruit and determine the market price for each grade. This may show a greater cash return to the grower than if total yield alone were the only criterion used. Return bloom treatments may also inhibit vegetative growth, and an additional benefit may be a reduction in the amount of pruning necessary. There is also a possibility of delayed bud growth in the spring which can be helpful to the grower in areas of late spring frosts.

Some other effects which should be reported may include; that the treatment can hasten or retard the maturity of the crop in the treatment year; and/or the treatment may increase the severity of a physiological disease known as "bitter pit." Factors affecting chemical treatments to control alternate bearing in apples and methods of testing are discussed in the references listed below.

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F. Control of sex expression in cucumbers and squashes. The biochemical control of sex expression in cucmbers and squashes aids in the production of hybrid seed and also can be useful in increasing yield, especially where once-over destruct harvest is practiced. A chemical treatment is made at the early seedling stage of growth to increase the number of pistillate (female) flowers and to decrease the number of staminate (male) flowers. These treatments bring about formation of female flowers at lower nodes where normally only male flowers are formed in the standard (monoecious) cucumber and squash varieties. Factors affecting this sex response and methods of testing to demonstrate the efficacy of a chemical treatment have been discussed in a number of publications (McMurray and Miller, 1968 and 1969; Robinson et al., 1969; Rudich et al., 1969; Sims and Gledhill, 1969; Weaver, 1972).

1. Test conditions. For sex ratio studies, field trials consisting of single-row plots 8 ft. (2.4 m) long with 10 plants per plot are adequate. Each treatment and control plot should be replicated 4 times. For yield trials, however, single-row plots 40 ft (12.2m) long with 120 plants per plot should be used, with 6 replications per treatment. The test plots should be located in the middle of a field containing sufficient untreated monoecious plants to assure an adequate supply of pollen. Application should be made to seedling plants to assure good coverage (100 gal/acre) (1/ha).

To evaluate the effectiveness of a treatment on sex expression, the sex of each flower at each node should be determined on a weekly basis during the blooming period. To determine the effect on yield, the plots should be harvested twice a week, starting when the first fruit is at the proper stage. For mechanical harvesting, each plot should be harvested separately at the correct stage to take into account any delay or hastening of maturity. In all cases, the yield should be separated by grade, and the market price of each grade at each harvest date should should be determined.

2. Additional test conditions and variables that should be studied.

a. For hybrid seed production, the amount of seed per fruit should be determined. Also, the percentage germination and the storage life should be studied.

b. The response between varieties can differ significantly, and the correct timing, rate, and number of applications should be studied on each variety under different environmental conditions.

c. The effect on delay or hastening of maturity should be determined.

d. Effects on fruit quality and fruit shape should be made.

e. Since the effect on sexual modification toward femaleness in the treated plants may be temporary, studies should be made to determine the length of effectiveness of each treatement.

f. The effect of rainfall following application should be determined.

g. The effect of the treatment on vine growth should be noted.

h. Any effect on time of flowering should be noted so that the pollination line can by planted at the proper time to supply adequate pollen.

3. Interpretation of test results. To support claims as an aid to hybrid seed production, the data should show an increase in the percentage of female blossoms at the early stages of flowering. Seed production in the treated fruit should not be decreased and the hybrid seed should have good germination and storage life.

To support claims for increased yield, data are needed for yield and grade for the period during which the crop is harvested and marketed. Total yield during the growing season is often very misleading, especially if a large percentage of the fruit falls in the low-priced grades. In once-over destruct harvests, comparisons are needed between yield from treated and untreated control plots harvested at the same stage of development. If the chemical treatment hastens or delays the development of the fruit, it may not be necessary to harvest the treated and untreated control plots at the same stage of development, but perhaps at different times. The chemical treatment should not cause any adverse effect on fruit shape or quality.

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Sims. W.L., B.L. Gledhill, and B.J. Hoyle. 1969. Effect of Ethrel on Yield of Pickling Cucumbers, Field Experiments - 1969. Univ. of Calif., Davis, Ext. Release No. 129. 4 pp. (Nov. 1, 1969).

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G. *Control of fruit maturation and ripening*. Plant regulators are being tested experimentally and used commercially to achieve a number of specific objectives related to fruit maturation and ripening. Included in these objectives are:

(1) Enhancing pigmentation in cherries, cranberries and apples (Chaplin and Kenworthy, 1970; Eck, 1969; Edgerton and Blanpied, 1968);

(2) Hastening or delaying maturity in peaches, tomatoes, and apples (Looney, 1972; Robinson et al., 1968; Unrath, 1971);

(3) The concentration of ripening in tomatoes, blueberries, and apples (Iwahari and Lyons, 1970; Eck, 1970; Schomer et al., 1971);

(4) Improving uniformity of ripening and enhancing fruit quality of bananas (Russo et al., 1968); and

(5) Enhancing abscission and loosening fruits to facilitate mechanical harvesting (Bukovac et al., 1969, 1971).

1. *Test conditions*. Test conditions that should be reported are plot sizes, number of plants per plot, and number of replications and controls. Information on product application should include: number of treatments; rates of application; time of application; methods of application; a comparison of dilute versus concentrated spray applications and low versus high gallonage applications; and possible interaction with other chemicals applied. Differential varietal responses are important and should be adequately investigated. Possible parameters in measuring growth regulator effects on the maturation and ripening process incude such quality measurements as sugars, acids, pigments, viscosity, firmness, dry weight, flavor, appearance, short- and long-term storage quality, and size. Yield, also an important parameter, might include measurement of total yield, marketable yield, grade, percent

of crop mature at a single harvest, and yield distribution over the entire harvest period. Residual effects should also be evaluated, particularly as they may influence subsequent flowering and fruit set on perennial plants and seeds of annuals.

2. Other conditions that should be studied.

a. Plant condition should be monitored, noting particularly any possible stress conditions.

b. Environmental conditions, particularly temperature, soil moisture, and possible adverse weather conditions (before, during, and after treatment), should be monitored.

c. Soil residual effects on greenhouse and container-grown crops should be measured.

d. Changes in insect and disease susceptibility should be noted.

e. Any chemical residue on the harvested fruit should be measured.

3. Interpretation of test results. Suggested interpretations in terms of the quantitative level of response obtained from plant regulator treatment might include:

a. A 15% improvement in yield, when yield is the primary objective;

b. Advance or delay of harvest by a week for a plant regulator treatment designed to modify the harvest season of particular crop;

c. A pigment enhancement of 50%, particularly in early harvested fruit; and

d. Concentration of yield which results of 80% of the total harvest occurring in a single picking for a plant regulator designed to concentrate the harvest.

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Schomer, H., M.M. Williams, and H.D. Billingsley. 1971. Effect of combinations of growth regulators on maturity and quality of 'Tydeman's Red' apples. HortScience 6:453-455.

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H. Control of fruit abscission. Soon after mechanical harvesting of fruits was tried, it became obvious that, in many cases, treatment of the trees or vines with fruit abscission agents (chemical looseners) would be beneficial, and that, with some types of mechanical harvesting equipment such as air harvesters, it is absolutely necessary (Wilson and Coppock, 1968; 1969). Citrus, cherries, olives, apples, grapes, and pecans are examples of crops whose harvest can be aided by the use of such compounds (Weaver, 1972). Specific requirements for each crop may vary somewhat, but the overall principles involved appear to be similar. Although the following test conditions relate primarily to citrus fruits, some examples of problems encountered with other crops area also included.

1. Test conditions.

a. Method of application: The application method usually will be by dilute or concentrated spray. Most of the current abscission chemicals under investigation appear to function through contact rather than systemic action. A uniform spray coverage of trees and vines, therefore, is necessary. Other methods of application should be evaluated as to their practicality under field conditions (Wilson, 1966; 1967).

b. Timing and environmental conditions: Most abscission agents produce fruit loosening within 3 to 7 days following spray application, although this may vary depending on the crop involved. With citrus, the abscission agent can normally be applied at any time during the period when the fruit is considered legally mature (Wilson, 1969).

Weather conditions may affect abscission chemicals (Wilson, 1969; 1972) and should be studied. Although no loss of activity due to high temperatures has been reported, abnormally wet and cold conditions may adversely affect results either by washing off the material before it has time to act or delaying or reducing its effects by slowing metabolic processes.

c. Consistency of performance: Failures caused by adverse environmental conditions can occasionally be expected; unexplained failure occurring under good environmental conditions, however, should be rare. Thus, inconsistency in chemical performances should usually be interpreted unfavorably. The effects of various root stocks should also be considered in evaluating results, since, with citrus fruits, certain rootstocks produce fruit which loosen more readily when abscission chemicals are applied (Cooper and Wilson, 1971).

d. Physical measurements of chemical effectiveness: The most common method used to determine degree of citrus fruit loosening is through use of a pull-force measuring device (Hendershott, 1964). Most of these devices measure straight pull-breaking force necessary to separate the stems from the fruit. These pull forces may vary from a few grams for olives to 30 to 40 lb (13.6 to 18.1 kg) for some citrus fruits. A measure of effectiveness is a substantial reduction in the average pull-force measurements from that of a control.

2. Interpretation of results. In evaluating abscission results, two principal factors must be considered, depending on which of the methods of removal discussed below is employed and the ultimate use of the fruit (processing or fresh).

If fruit is to be shaken to the ground for mechanical or hand pickup, maximum fruit loosening is desired. Under these conditions, there is a high probability that substantial fruit drop will occur before harvest (Wilson, 1973). With citrus fruits, pull force will be reduced from 14 to 18 lb (6.4 to 8.0 kg) for untreated fruit to an average of 2.5 lb (1.1 kg) for treated fruit. Fruit drop of 20 to 50% could be expected when fruit is this loose. Under some experimental conditions and sometimes under field conditions, fruit loosening will be so complete that the fruit will separate from the stem when touched and prevent the measurement of pull force. These results can be classed as "too loose to pull," "all fruit dropped," or some other appropriately descriptive phrase.

If fruit is to be shaken by a mechanical device onto a catchframe, fruit loosening should not be as complete as above. A chemical developed for use with catchframes should loosen citrus fruit ideally to no less than 6 lb (2.7 kg) average pull force.

Abscission agents are of two types: (1) chemicals which release ethylene or cause ethylene production by the fruit without peel injury; and (2) chemicals which may cause superficial rind injury with resulting wound ethylene formation. Although abscission not related to the action of ethylene may be possible, all present commercial abscission agents appear to utilize the ethylene mechanisms. Many fruits, including citrus fruit intended for fresh fruit utilization, apples, cherries, and (perhaps) olives and pecans respond to chemicals with this mode of action.

Citrus fruits loosen readily when chemicals cause superficial peel injury. Chemical damage to the rind is acceptable on citrus fruit for processing if it does not cause fruit splitting and excess rotting under wet conditions (Wilson, 1972) or adversely affect juice flavor.

The amount of phytotoxicity which can be tolerated by trees and vines before yields and/or fruit quality are adversely affected varies with the crop and time of year. No specific percentages of leaf drop have been determined for citrus fruits which indicate the upper limits of phytotoxicity. Experience has shown that a fairly large margin of safety is necessary. With a crop such as apples, excessive leaf drop accompanying abscission sprays applied in the fall occurs then anyway. With some other crops, economic considerations may be such that some phytotoxicity can be tolerated.

Because mature fruits, leaves, immature fruits, and flowers are all subject to abscission through basically the same physiological process, the plant regulator(s) selected for abscission should be specific to removal of only mature fruits or nuts. The 'Valencia' orange, for example, poses a distinct harvesting problem because its lengthy development period (11 to 18 mo) results in the presence of flowers of immature (green) fruits during most of the harvest season. It is important, therefore, that any abscission chemical selectively loosen mature fruit but cause minimum phytotoxicity to flowers, immature fruits, or young growth which might be present.

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I. Increased berry size of seedless varieties of table grapes. When vines of Thompson or other seedless grape varieties are girdled at the fruit-set stage, a large increase in berry size -- sometimes as much as 100 percent -- usually results. Since about 1960, almost all Thompson Seedless grapes intended for table use have been sprayed with 1 or 2 applications of the plant regulator gibberellin. These treatments, along with girdling, increase berry size in a uniform manner (Weaver, 1972).

1. Test conditions. The test chemicals are usually applied after shatter (separation of calyptra from flower) following bloom (also the normal girdling time). Four vines per replicate should be sprayed to wet cluster area thoroughly. Use 5 concentrations of the test product and compare results with untreated controls and with gibberellin at label rates.

2. Test results. At harvest the following measurements should be made:

a. Crop weight per vine.

b. Average weight of 100 berries per vine to indicate berry size. (Take berries from various cluster and from base, middle, and apex of cluster.)

c. Brix value of juice taken with a refractometer.

References.

Stewart, W.S., D. Halsey, and F.T. Ching. 1958. Effect of the potassium salt of gibberellic acid on fruit growth of Thompson Seedless grapes. Proc. Am. Soc. Hort. Sci. 72:165-169.

Weaver, R.J. 1972. Plant Growth Substances in Agriculture. W. H. Freeman & Company: San Francisco. Pp. 349-350.

J. Loosen compact-clustered varieties of Vitis vinifera (wine grapes). The incidence of rot in seeded wine grapes is excessive in cultivars (varieties) that produce compact clusters, for example, 'Zinfandel' and 'Carignane'. Berries are pushed off by other expanding berries, and the juice from the fallen fruit fosters decay organisms. Tight clusters are also slow to dry after rains. Loosening of the cluster is a result of reduced set, cluster elongation, and/or production of shot berries (Weaver, 1972); prebloom sprays of the plant regulator gibberellin also loosen these clusters (Weaver and McCune, 1959).

1. Test conditions. Test chemicals are usually sprayed onto the vines 2 to 3 weeks before bloom when the shoots are 15 to 20 in. (38.1 to 50.8 cm) long; clusters should be 3 to 4 in. (7.6 to 10.2 cm) in length, but may range from 2 to 5 in (5.1 to 12.7 cm) in length. Use 4 replicates of 4 vines per treatment. Use 5 concentrations of the test product and compare results with untreated controls and gibberellin at label rates for the variety. The clusters must be thoroughly wet.

2. Test results. At harvest the following measurements should be made:

a. Estimate of looseness percentage. The compact control clusters are considered to have 0 percent unoccupied space. Estimate that part of the total volume of a cluster that is not occupied by berries.

b. Number of berries per cluster (for the determination of effect on berry set).

c. Length of cluster from basal lateral to the apex of the cluster.

d. Berry size (average weight of 100 berries).

e. Degrees Brix value of juice taken with a refractometer.

f. Percentage of rot which has occurred in each treatment.

References.

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K. Acidity reduction in citrus. The two main purposes for acidity reduction in citrus fruits are to produce early maturity of fruit and thereby lengthen the marketing season, and to produce sweeter fruit. In analytical chemical terms, "sweet" means "high ratio fruit" as measured by the standard accepted Brix/acid determination. Maturity of citrus fruits generally follows the pattern of gradual reduction of acid during the fruit season, and usually there is a corresponding increase in soluble solids (sugars). Certain plant regulators are used to induce acidity reduction in citrus fruits.

1. Test conditions. Field trials to reduce acidity may consist of single-tree plots replicated 6 times. Materials are applied as sprays to thoroughly cover fruit and foliage. With citrus fruits which maintain a generally recognized low respiration rate, the time of application of the sprays is usually 4 to 10 months before harvest. (The development of sprays which would shorten this interval would be desirable.) At the present time, maturity sprays are applied from postbloom until the end of the summer spray period, although the most effective time is 1 to 6 weeks following bloom -- late March to early May.

Twenty fruit per tree, a total of 120 fruit per treatment picked at random (inside and outside of the canopy), are taken from the treated, control, and standard product trees at biweekly intervals 30 days before and during the normal harvest period. The following measurements should be taken:

a. Determine the % juice for each sample (total weight of squeezed juice per unit weight of fruit).

b. Determine the % acid (total titratable acid) using phenolphthalein as the indicator and 0.3125 N NaOH as the basic solution.

c. Determine Brix value for each sample.

d. Calculate ratio of Brix value to acid.

2. Interpretation of test results. At least 2 limitations to use of an acidity-reducing compound should be considered:

a. Oranges, tangerines, grapefruit, tangelos, and tangelo hybrids can be subject to excessive acidity reduction and produce a sweet fruit with an undesirable "insipid" flavor.

b. "Flavor" of a fruit or fruit juice is made up of several components. Although a plant regulator may reduce juice acidity of immature fruit to what would be considered acceptable for mature fruit, it would not affect the raw, immature flavors also present in the immature fruit. Normally these immature flavors diminish at a Brix value of 9.0 or higher.

L. Apple scald inhibitors. Substantial information about scald, a surface disfigurement particularly severe in apple fruits, is available, as this problem has been widely studied for over 60 years. (Huelin, 1965; Smock, 1961; Smock and Southwick, 1945). The cause is still unknown or indefinite. Because it is usually much worse on early pickings, it is associated with immaturity. Scald susceptibility varies from season to season, so weather before harvest also is a factor. Many researchers believed that volatile materials caused scald, since relatively high levels of esters induce an artificial scald-like injury on apples; but this volatiles theory is no longer widely accepted. Recently, Australian researchers (Pierson et al., 1971) have suggested that oxidation products of alpha-farnesene are the direct cause of superficial scald. They further suggest that effects of artificial antioxidants on the oxidation of alpha-farnesene could first be measured in comparatively short tests before testing for control of scald on apples.

For many years, use of tissue wraps containing mineral oil around each fruit was the standard scald-control procedure. Plant regulating substances, such as naphthaleneacetic acid (NAA) applied as a post-harvest dip, provided considerable control but were not used commercially (Schomer and Marth, 1945). Commercial scald control is now obtained by using 1 of 2 antioxidants (Smock, 1961): diphenylamine, and 1,2-dihydro-6-ethoxy-2,2,4-trimethylquinoline (ethoxyquin). Both chemicals are registered for use on apples and provide good scald control (Dewey and Dilley, 1964; Hardenburg and Anderson, 1962; Huelin, 1964; Mattus and Rollins, 1968).

1. Test conditions.

a. Use freshly harvested applies, 50 to 100 replicate. More scald will develop in control lots if fruit is picked 1 week before the best commercial date. 'Stayman Winesap', 'York Imperial', 'Rhode Island Greening', 'Grimes Golden', 'McIntosh', 'Rome Beauty', and 'Red Delicious' are scald-susceptible cultivars.

b. Apply test product and appropriate standard products within the first 7 to 10 days after harvest. Dip or submerge apples for 10 sec. in test solutions or suspensions. Keep test suspensions well-agitated.

c. Fruit temperature and treatment solution temperature should be moderate (60° to 70°F) (15.6° to 21.1°C). Cold fruit will not take on a good coating of test chemicals.

d. After treating fruit, tilt boxes or turn fruit to improve drainage and prevent possible chemical burn in stem or calyx cavities. Examine for initial visible residue.

e. To predict scald intensity, store treated and untreated fruit samples in boxes with unsealed 1.5-mil polyethylene liners for 4 to 6 weeks at 70°F (21.1°C (Smock, 1961).

f. To determine commercial scald control, store treated and untreated fruit samples in unlined boxes for 5 to 6 months at 32°F (0°C)[38°F (3.3°C) for 'McIntosh'] with high relative humidity (90-95%), then transfer to 70°F for 7 days to allow scald symptom development (Hardenburg and Anderson, 1972; Huelin, 1964; Mattus and Rollins, 1963; Smock, 1961).

2. Interpretation of results.

a. Determine the number of fruit with no scald, slight scald, and severe scald. Slight scald is that affecting 10% or less of the skin surface and is light brown in intensity. Severe scald is that affecting more than 10% of the surface or is dark brown and markedly disfiguring.

b. Determine flesh firmness of fruit from treated and control fruit samples using a fruit pressure tester to determine any adverse effect on ripening or softening rate.

c. Examine fruit for "off odor," "off flavor," visible residue, lenticel injury or chemical burn symptoms.

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III. Desiccants

Desiccants are intended to be applied to desirable plants or crop plants for the purpose of altering plant parts by inducing or accelerating the drying of plant tissues. The methods of evaluation of desiccation vary with both the crops and investigators. Many investigators have developed valid but varied techniques for evaluation of usefulness. For registration purposes, testing under actual field conditions is currently the only acceptable method for producing data for evaluating the usefulness of potential desiccants. New protocols for evaluating desiccants are continually being developed because both new uses and new chemicals are being sought. In all cases, however, data obtained from field tests must support the label claims for the product and must be representative of the results that the producer can expect when the product is used in accordance with label instructions under commercial conditions. The references presented are not intended as an exhaustive list of methods, since the purpose of desiccation vary from crop to crop, and many valid procedures exist. Referenced test methods are presented for the crops on which desiccants are presently used.

References.

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IV. *Defoliants*.

Defoliants are intended to be applied to desirable plants or crop plants for the purpose of removing leaves by inducing or accelerating leaf abscission. The methods of evaluation of defoliation vary with both the crops and investigators. Many investigators have developed valid but varied techniques for evaluation of usefulness. For registration purposes, testing under actual field conditions remains the only acceptable method for producing data for evaluating the usefulness to potential defoliants. New methods and protocols for evaluating defoliants are continually being developed because both new uses and new chemicals are being sought. In all cases, however, data obtained from field tests should support the label claims for the product and must be representative of the results that the producer can expect when the product is used in accordance with label instructions under commercial conditions. The references presented are not intended as an exhaustive list of methods, since the purposes of defoliation vary from crop to crop, and many valid procedures exist. Reference test methods are presented for the crops on which defoliants are presently used.

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Series 95: EFFICACY OF INVERTEBRATE CONTROL AGENTS

§ 95-1 General considerations.

(a) Overview. A wide variety of pesticide products are used for invertebrate control. These products are used in households, industry, and agriculture, as well as in public health programs. They are applied against a tremendous variety of pests by many different methods in numerous kinds of formulations. Sections 95-1 through 14 provide guidance related to efficacy test methods generally adequate to develop acceptable data on invertebrate control pesticides.

(b) Applicable sections. (1) This section. The considerations contained in this section apply to all studies in the series, from §§ 95-2 through -14, unless one of these sections contains a specific consideration on the same subject. In such a case, the specific consideration in the individual section shall apply to the conduct of that particular study.

(2) Sections 95-2 through -14. Each of these sections contains specific considerations related to the performance of studies for one group of invertebrate control pesticides. These sections also provide suggested performance standards, which are levels of pest control that should be achieved through use of the pesticide product. These suggested performance standards are to be used for guidance purposes only. [Also see paragraph (d)(5) of this section.]

(3) Section 90-2. Section 90-2 establishes general considerations for evaluating the performance of invertebrate control products.

(4) Hazards to non-target organisms. Subdivision E - Hazard Evaluation: Wildlife and Aquatic Organisms, and Subdivision J - Hazard Evaluation: Nontarget Plants, and Subdivision L - Hazard Evaluation: Nontarget Insects contain requirements for data on the hazards to non-target species which might result from use of a product. Sections in these two subdivisions provide guidance on the kinds of information which must be reported if adverse effects on non-target organisms are observed during an efficacy test.

(c) Definitions. The following definitions apply to all sections of series 95 of this subdivision:

(1) An "invertebrate control pesticide" means any pesticide product which is intended for preventing or inhibiting the establishment, reproduction, development or growth of, destroying, repelling or mitigating invertebrate animals declared to be pests 1/, including any member of the

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1/ The pest names used in this unit on invertebrate control agents conform to those listed in the "Common Names of Insects and Related Organisms" published by the Entomological Society of America, 4603 Calvert Road, Box AJ, College Park, Maryland 20740, and "The EPA List of Insects and Other Organisms", Seventh Edition, published April 1982. Contact the Index Section, ASIB-BFSD (TS-768), of the Agency for the latest update on invertebrate pest names in the EPA listing.

Class Insecta, other allied classes in phyla Arthropoda, Annelida, and Mollusca, such as slugs and snails, but excluding nematodes. [Refer to § 162.3(ff)(10) and (11) of the FIFRA Sec. 3 regulations.]

(d) General considerations. (1) Efficacy data should be derived from testing conducted under conditions typical of actual or proposed use, or, where applicable, under controlled laboratory conditions which simulate actual use.

(2) The test substance should be the formulated product evaluated at various dosage levels including those dosage rates associated with its proposed use. It should be tested under all techniques intended to be used in applying the product.

(3) Data on the compatability of the test substance with other substances will be developed in accordance with § 90-2(a)(3)(i) through (vi), if the test substance will be used in sequence or with another substance.

(4) Data should demonstrate the effect of the test substance on various life stages of pests, host plants or animals, fruit finish, yield, palatability, marketability, carcass grade, or other significant factors. The data should clearly establish the method of action of the test substance in repelling, destroying, or mitigating pests.

(5) The efficacy of the test substance should be established with reference to the applicable suggested performance standard.

(i) The suggested performance standards contained in the following sections are generally stated in terms of percent control, based on a comparison of treated organisms and untreated control organisms. In certain situations, the test substance may be evaluated in comparison to a product of known efficacy. Under some other circumstances, the performance standards are expressed as acceptable levels of damage.

(ii) The conditions under which the suggested performance standards apply are listed generally in § 90-3(c) and the following sections. These performance standards are not intended to be absolute or inflexible.

(iii) An analysis of variance and multiple range test or other appropriate statistical analysis should be conducted to determine the reliability of data, when a question of relative effectiveness occurs.

(6) Dose response data should accompany applicable site/pest crop combinations. The benefits such as increased yield, unblemished fruit, reduction in nuisance pest levels, to be derived from each dosage rate to be registered for control of a particular pest should be clearly defined and reported. Dose response data for crops other than the pesticide site/pest combination will be considered if submitted and referenced.

(e) Use of § 95-30 references. Section 95-30 (a) and (b) and contains references to acceptable test protocols for evaluating the efficacy of invertebrate control agents when used in the kinds of treatments described in §§ 95-2 through -14. The references contain criteria covering areas such as test design, site selection, sample size, plot selection and management, application or treatment techniques and equipment, dosage selection, levels of infestation (pest population), examination times, sampling and evaluation procedures, statistical analysis information and reporting procedures.

§ 95-2 Foliar treatments.

This section is concerned with efficacy test methods for invertebrate control agents applied as foliar treatments to deciduous tree fruits, small fruits, citrus and subtropical fruits, tree nuts, field crops, forage crops, rangelands, and vegetable crops for protection from economic injury by insects, mites, and certain other invertebrate pests.

(a) General considerations. The testing of a pesticide product for use on food crops in home gardens should follow the same test procedures as are appropriate for evaluating the product's commercial use, except that tests with home use products should also include dosage levels higher than the label-recommended rates.

(1) Site selection. Crop plantings should be selected which are representative of cultivars (varieties), ages of plants, cultural practices, and pest populations commonly encountered. For general considerations, refer to § 90-2(a)(3)(iv). When selecting test sites, other factors such as climate; texture, composition, and type of soil; and geographic location should be considered. For general considerations, refer to § 90-2(b)(1) through (3).

(2) Plot size. Plots of a size suitable for commercial applications should be used. These should have dimensions large enough to avoid any effects from drift onto untreated control and sampling areas. It may be necessary to use several planting sites in the same immediate area to deliver a form of replication where field dimensions preclude internal replicate design.

(3) Number of trials. A minimum of 5 large-scale, geographically-separated trials (e.g., when commercial application equipment is used) are generally recommended, but the number of trials can vary somewhat due to the accessibility of infestations, fluctuations in pest populations pressures, behavior, insect resistance, and other important characteristics of the target pest. The resistance problems may make it necessary to test in certain geographic areas (e.g., greenbug on sorghum should be tested in Trans-Pecos if a national label is requested).

(4) Application techniques and equipment. Data should be developed to support conventional ground, aerial, or ultra-low volume applications and crop/pest stage. For general consideration, refer to § 90-2(a)(3)(ii).

(5) Evaluation and reporting procedures. The procedures used to evaluate product performance should be specified. Reports should, include for example, yield information, insect counts, percent damage, percent marketable fruit, percent russeting, extent of catfacing, or other measures for determining the effectiveness of the test substance. Results should include the average infestation reductions throughout the sampled post-treatment period compared with that in control plots or in preand post-treatment periods. Raw data on pests counts, injury counts, or other measures of control should be summarized. Any adverse effects such as phytotoxicity, staining, discoloration, and other factors should be reported. For general considerations, refer to § 90-2 (c)(1) through (3) and § 90-2 (d)(1) through (4).

(b) Suggested performance standards. Unless otherwise specified, these standards are presented on the basis of pre- and post- treatment pest population counts from treated as well as untreated plots and standard comparative teatments. All percentages of control refer to the performance of the test substance (as determined by insect counts, yield data, and any measures correlated to insect population pressures) against the vulnerable stage(s) of the target pest, when evaluated according to a recognized treatment program under actual field conditions.

(1) Fruit and nut crops. (i) Pome and stone fruits. (A) Apple maggot and other fruit flies. A minimum of 80% insect population reduction (control), based on percent infested fruit, determined by counts of ovipositional punctures and/or tunneling, made throughtout the growing season and at harvest. Counts should include both dropped and harvested fruit.

(B) Aphids and leafhoppers. A minimum of 90% insect reduction. Dormant treatments should give complete control of aphid eggs.

(C) Catfacing insects. A minimum of 90% control, based on evaluation of catfacing injury of fruit prior to and at harvest.

(D) Major chewing insects. A minimum of 95% control of codling moth and redbanded leafroller, and 80% control of tufted apple bud moth based on percent fruit injury determined by counts made throughout the growing season. Counts should include both dropped and harvested fruit.

(E) Foliage feeding mites. A minimum of 80% control where predatory mites are present. A minimum of 99% control when no predatory mites are present. Dormant treatments should give complete control of mite eggs.

(F) Scale insects. A minimum of 90% control, based on pretreatment counts of live females before pesticide applications, and post-treatment counts following the last pesticide application. Dormant treatments should give complete control based on pre- and post-application counts.

(G) Twig borers on stone fruits. The twig borers include the oriental fruit moth and the peach twig borer. A minimum of 90% control, based on counts of the number of damaged terminals per tree and percent injured fruit. Data should include recorded percent injury from both dropped and harvest fruit.

(H) Plum cruculio. A minimum of 90% control, based on percent infested fruit, determined by counting egg-laying scars on fruit samples and number of larvae per 100 dropped fruit collected throughout the growing season and at harvest.

(I) Wood-boring insects. A minimum of 85% control determined from pre-treatment poplulation estimates, and detailed examinations of the trunk and larger limbs in the fall following the application of pesticide treatments.

(ii) Small fruits. (A) Grape insects. A minimum of 98% control of grape berry moth, based on the comparison of injured berries for treated, standard, and untreated plots. A minimum of 95% control of grape leafhopper complex, based on pre- and post-treatment population counts on the number of leafhoppers per leaf.

(B) Blueberry insects. Almost complete (99%) control of blueberry maggot, based on larval counts obtained from post-treatment examinations.

(C) Strawberry insects. A minimum of 95% control of strawberry leafroller, based on pre-treatment and post-treatment counts of the percentage of leaves damaged.

(D) Cranberry insects. A minimum of 95% control of cranberry fruit worm and cranberry tipworm, based on percentage of infested berries from treated and untreated plots.

(E) Raspberry insects. A minimum of 95% control of raspberry fruit worm and raspberry crown borer, based on comparison of injured fruits and crowns in treated and untreated plots.

(F) Currant insects. A minimum of 90% control of currant borer based on the number of injured shoots in treated and untreated plots. A minimum of 95% control for currant fruit fly and currant aphid. Currant fruit fly infestation is evaluated by examining berries to determine the number of larvae and/or pupae in treated and untreated plots. Aphid control is determined by pre- and post-treatment aphid counts.

(iii) Citrus and subtropical fruits. (A) Armored scale insects. A reduction of 80% infestation over pre-treatment, based on 90-day post-treatment counts, and 50% infestation reduction over pre-treatment based on 12-month post-treatment counts.

(B) Soft scale insects. A reduction of 50% infestation over pre-treatment, based on 12-month post-treatment counts.

(C) Other homopteran insects. A minimum of 95% control for whiteflies and 80% control for mealybugs. Almost complete (99%) control for aphids, based on an initial post-treatment count and 90% control based on a 14-day post-treatment count.

(D) Miscellaneous groups or species. No more than 10% scarred fruit from thrips and less than one thrips per terminal, based on counts taken within 4 weeks after treatment. Less than 15 orangeworm egg masses per tree, based on per hour search basis counts. A minimum of 90% control for fruitflies.

(E) Acari. A minimum of 80% control for all species of mites except the citrus rust mite which should be almost complete 99% control of the vulnerable stage based on 4-6 week post-treatment counts.

(iv) Tree nuts. (A) Armored scale insects. A minimum of 80% control for all species, based on pre-treatment counts and post-treatment counts taken not less than than 40 days after each treatment.

(B) Soft scale insects. A minimum of 80% control, based on pre-treatment counts of the number of live scales on terminal growth or the number of live scales on a circular area 15 mm in diameter randomly selected along the midrib of each leaflet.

(C) Other homopteran insects. A minimum of 80% control of aphids, based on pre- and post-treatment counts and expressed as aphids per leaflet. A minimum of 90% control for spittlebugs, based on pre- and post-treatment counts and expressed as the percent reduction in the number of terminals infested with live spittlebug nymphs.

(D) Lepidopteran insects. Less that 5% infested nuts at harvest for codling moth and filbertworm. Less than 30 infested fruit spurs per 300 fruit spurs per 6 tree subplot, based on post bloom counts for fruittree leafroller. Harvest count should be less than 10% injured fruit for fruittree leafroller. Less that 10% infested shucks or a 90% increase in the number of nuts over number in untreated controls for hickory shuckworm. Less than 20% damaged kernels for naval orangeworm. A minimum of 80% control for peachtree borer. Less than 15 worm-damaged terminals per count tree and less that 10% worm-damaged harvested fruit for peach twig borer. Over 80% retention of nuts on the tree indicates adequate control of pecan nut casebearer. A minimum of 80% control for pecan serpentine leafminer, and 90% control for western tent caterpillar.

(E) Miscellaneous insects. Less than 15% damaged nuts, based on harvest counts for the walnut husk fly and pecan weevil.

(F) Acari. A minimum of 80% control of European red mite and other tetranychid mites. Less than one mite per leaf during 45 day post-treatment counts for Pacific spider mite and twospotted spider mite.

(2) Field crops. For the following insect pests of corn, the data should indicate that a product demonstrates effective control which is equal to a better than other product of known efficacy used as reference standards. Efficacy is based upon:

(i) Corn. (A) Southwestern corn borer. Percent stalk girdling, damage to harvested ears and yield.

(B) European corn borer. For leaf-feeding first-generation European corn borers, a visual plant rating damage scale of 1-9; for second-generation European corn borers, number of feeding tunnels determined by stalk evaluations, borer damage to harvested ears, and yield.

(C) Corn earworm. United States standards for U.S. Fancy Grade fresh sweet corn permit no more than 10% damaged ears from all sources. Efficacy is based on percent of injury-free ears, percent of worm-free ears, or percent of worm-infested ears.

(D) Fall armyworm. Percent of injury-free or worm-infested plants from observations made 1-7 days after the last insecticide applications.

(E) Corn rootworm adults. Adult counts are made pre-treatment and 1, 3, 7 and 14 days following applications. Soil core samples are taken after the first killing frost, washed through custom-built corn rootworm egg extractors, and the number of corn rootworm eggs counted. Egg counts are used to estimate insecticide effectiveness in reduction the infestation.

(ii) Cotton. (A) Boll weevil. Based on percent damaged squares or bolls per acre (hectare) correlated with subsequent harvest yield data.

(B) Bollworm and tobacco budworm. Based on percent damaged squares or bolls per acre (hectare) correlated with subsequent harvest yield data.

(C) Lygus bugs. A minimum of 85% control correlated with subsequent harvest yield data.

(D) Mites. A minimum of 80% control, based on pre- and post-treatment population counts.

(E) Pink bollworm. The data should indicate that a product demonstrates effective control which is equal to or better than other products of known efficacy used as reference standard which are determined by adult moth counts from pheromone traps or weekly cutting and examination of bolls.

(iii) Peanuts. (A) Cabbage looper, green cloverworm, bollworm. A mimimum of 80% control.

(B) Lesser cornstalk borer. A minimum of 90% control based upon a reduction in the number of infested vines and/or pegs; or a 90% reduction in the number of infested sample sites for whole-field applications where pre-treatment data indicate a minimum of 30% of the sample sites as infested. Those reductions should further correlate to yield increases as compared to untreated sites to confirm the benefits of pesticide application.

(C) Granulate cutworm. The data should indicate a minimum of 90% control based upon population counts (usually expressed as larvae per row ft.) and reductions in damaged vines.

(iv) Sorghum. (A) Greenbug. A minimum of 90% control, based on pre- and post-treatment insect counts, correlated with yield data.

(B) Sorghum midge. The data should indicate that a product demonstrates effective control which is equal to or better than other products of known efficacy used as reference standards. Population infestation is determined by estimating the number of sorghum midges for a number of sorghum heads. Level of sorghum midge infestation are determined by sorghum midge damage to individual florets. Yield data is considered to be the most appropriate method of evaluating insecticide efficacy against the sorghum midge.

(C) Banks grass mite. Control is determined by pre- and post-treatment counts of all life stages of this pest. Plant lodging data is considered to be indicative of product efficacy of this part. Plant product-damage and/or lodging data are also considered indicative of product efficacy when correlated to mite counts.

(v) Grain crops: greenbug. A minimum of 90% control, based on pre- and post-treatment insect counts, correlated with yield data.

(vi) Soybeans. (A) Soybean podworm, stink bugs. A minimum of 80% control for soybean podworm and stink bugs, based on post-treatment counts of live insects, usually 48 hours after treatment. Yield data aids in supporting claims involving timing of applications according to economic thresholds.

(B) Mexican bean beetle, bean leaf beetle. A minimum of 85% control, based on pre- and post-treatment insect population counts. An estimation of insect damage on a scale of 0 - 100% is appropriate for evaluating single applications, but not multiple applications.

(C) Southern green stink bug. A minimum of 80% control based on post-treatment insect counts of number of dead and living fourth instar southern green stink bugs in artificially-infested caged tests.

(vii) Sugarbeets: beet armyworm, fall armyworm. A minimum of 85% control, based on pre- and post-treatment insect counts.

(viii) Sugarcane: sugarcane borer. A minimum of 80% control, based on the number of live and dead larvae in post-treatment counts, number of bored points, and number feeding in the leaf sheath.

(ix) Sunflowers: sunflower moth. A minimum of 80% control based on the evaluation of insect numbers and damage on a minimum of 100 heads collected after the last insecticide applicaion.

(x) Tobacco. (A) Tobacco budworm, tobacco hornworm, cabbage looper. A minimum of 90% control for all of these insects, based on pre- and post-treatment insect counts and leaf surface area damage.

(B) Green peach aphid, tobacco flea beetle. A minimum of 85% control. The performance evaluation against the green peach aphid is usually based on pre- and post-treatment counts of insects per leaf. The performance evaluation for tobacco flea beetle is usually based on the number of holes in treated plants compared to the holes in a like amount of leaves from untreated check plants.

(xi) Wheat. (A) Greenbug. A minimum of 90% control, based on pre- and post-treatment insect counts coupled with yield data.

(B) Pale western cutworm. A minimum of 80% control, based on population reduction determined from soil samples evaluated at post-treatment intervals.

(3) Forage crops: alfalfa, red clover, birdsfoot trefoil. (i) Alfalfa weevil, Egyptian alfalfa weevil. A minimum of 80% control, based on pre- and post-treatment counts of adults and larvae from treated and untreated plots.

(ii) Spittlebugs. A minimum of 90% control, based on pre-treatment nymphal stage counts and post-treatment counts before the new adults appear.

(iii) Potato leafhopper, aphids. A minimum of 80% control, based on pre- and post-treatment counts correlated with a minimum of 90% control of hopper burn.

(iv) Seed chalcids, plant bugs. A minimum of 80% control. For seed chalcids, efficacy is evaluated by spliting seed pods at harvest to determine shriveled versus plump seeds. Efficacy against plant bugs is evaluated by counting nymphs in samples collected post-treatment in comparative treated plots.

(4) Rangelands. (i) Grasshoppers. A minimum of 90% control, based on population counts taken immediately prior to treatment (including dominant species and life cycle stage) and post-treatment counts at appropriate intervals, and compared to untreated control population fluctuations.

(ii) Range caterpillars. A minimum of 90% control, based on counts immediately prior to treatment (include all larval stages) and at appropriate post-treatment intervals estimated to embrace partial and maximum kill.

(iii) Harvester ants. A minimum of 90% control. Control evaluations should be made post-treatment at appropriate intervals and expecially in the spring of the year following application. Untreated check plots should be included.

(iv) Imported fire ants. A minimum of 90% control based on counts made for a minimum of 30 days for mound applications and 60 days for broadcast treatments. Percent control is based upon the percent reduction in both old and new active mounds in the treatment area as compared to precounts and untreated controls.

(v) Black grass bug complex. A minimum of 90% control, based on pre- and post-treatment population counts.

(vi) Chiggers. A minimum of 90% control, based on numbers of lesions per bird (turkey) pre- and post-treatment throughout the test period. Refer to § 95-8 (b)(vi). Grade of turkey as determined by Federal meat inspection also used as a criterion for determining efficacy.

(5) Vegetables (field grown). (i) Crucifers. (A) Cabbage looper, imported cabbageworm, diamondback moth, fall armyworm, beet armyworm, garden webworm, Hawaiian beet webworm, corn earworm. A minimum of 95% control, based on pre- and post-treatment larval counts supplemented with ratings of plant injury, yield records, counts supplemented with ratings of plant injury, yield records, and where appropriate, U.S. Grade crop evaluation ratings.

(B) Harelequin bug. A minimum of 95% control, based on pre- and post-treatment adult and larval counts, supplemented with counts of characteristic keg-shaped egg masses, plant conditions throughout the season either through ratings or actual counts of the harlequin bugs or their feeding scars, yield records, or unmarketable heads and their weights.

(C) Striped flea beetle, western striped flea beetle, western black flea beetle, sinuate striped flea beetle. A minimum of 95% control, based on pre- and post-treatment feeding scars on leaves. On seedlings, count the number of injured plants per foot of row.

(D) Aphids and thrips. A minimum of 90% control, based on pre- and post-treatment population counts of apterous aphids and thrips.

(ii) Cucurbits. (A) Striped cucumber beetle, spotted cucumber beetle, western spotted cucumber beetle, western striped cucumber beetle. A minimum of 99% control, based on pre-treatment counts of adult beetles, and post-treatment counts of adult beetles and fruit scars, correlated with yield data (weight of fruit) for at least 3 harvests.

(B) Squash bug. A minimum of 90% control, based on evaluation of pre- and post-treatment adult feeding injury and actual counts of nymphs per plot, coupled with yield data of marketable and non-marketable fruit (including number, weight, and injured and malformed fruit).

(C) Squash vine borer. A minimum of 90% control, based on counts of larvae, wilted plants, and borer tunnels, coupled with yield records.

(D) Pickleworm and melonworm. A minimum of 95% control based on counts of percent infested fruit throughout the season.

(E) Melon aphid. A minimum of 90% control, based on pre- and post-treatment melon aphid population counts coupled with foliage or tip injury and yield data including marketable and unmarketable fruit.

(F) Cabbage looper. A minimum of 85% control, based on pre- and post-treatment counts of the cabbage looper population coupled with yield data.

(G) Mites. A minimum of 95% control, based on pre- and post-treatment population counts.

(iii) Irish potatoes. (A) Colorado potato beetle. A minimum of 85% control, based on pre- and post-treatment population counts, coupled with yield data, including evaluation of potatoes according to U.S. Grades.

(B) Potato flea beetle. A minimum of 95% control, based on counts of pre-treatment feeding scars on leaves, post-treatment feeding scars on leaves, and larval injury to tubers, coupled with yield data and potatoes evaluated according to U.S. grades.

(C) Potato leafhopper. A minimum of 95% control, based on pre- and post-treatment population counts of nymphs, number of injured plants and diseased tubers, coupled with yield data and potatoes evaluated according to U.S. grades.

(D) European corn borer. A minimum of 75% control, based on number of plants injured by the European corn borer, number of borers per plant, coupled with yield data and potatoes evaluated according to U.S. grades.

(E) Potato psyllid. The test product should compare favorably with other products of known efficacy used as reference standards. Control is based on actual pre- and post-treatment counts of potato psyllid nymphs and infested plants, coupled with yield data pertaining to U.S. marketability grades.

(F) Potato aphid. A minimum of 80% control, based on pre- and post-treatment population counts of apterous potato aphids.

(G) Potato tuberworm. The test product should compare favorably with other products of known efficacy used as reference standards. The percent control achieved is based on counts of the number of tubers damaged at harvest time.

(iv) Lettuce: lettuce root aphid. The test product should demonstrate efficacy equal to or better than products of known efficacy used as reference standards. Percent insect control is based on comparative evaluation of root systems at harvest time to determine the presence of lettuce root aphid damage.

(v) Lima beans, snapbeans, southern peas. (A) Lygus bugs. The test product should demonstrate efficacy equal to or better than products of known efficacy used as reference standards. Percent insect control is derived from pre- and post-treatment population counts and percent *Lygus*-bug damaged beans at harvest.

(B) Bean leaf beetle. A minimum of 95% control, based on pre- and post-treatment population counts of the adult bean leaf beetle, at appropriate intervals supplemented by feeding hole counts, and inspection of roots for larval injury.

(C) Bean aphid. A minimum of 90% control, based on pre- and post-treatment population counts of all stages of the bean aphid.

(D) Cowpea curculio. The test product should demonstrate efficacy equal to or better than other products of known efficacy used as reference standards, based on counts of number of stings, feeding punctures, and damaged peas at harvest coupled with yield data estimating loss due to cowpea curculio injury.

(E) Potato leafhopper, beet leafhopper. A minimum of 90% control, based on pre- and post-treatment population counts, coupled with an evaluation of damage to foliage (potato leafhopper) and curly top damage (beet leafhopper).

(vi) Peas. (A) Pea aphid. A minimum of 90% control, based on preand post-treatment insect population counts.

(B) Pea weevil. (Reserved.)

(vii) Peppers. (A) European corn borer. A minimum of 95% control, based on European corn borer injury determined by pepper dissection and examination at harvest, coupled with yield data including percent marketable fruit, percent loss due to the European corn borer, and number of "rots."

(B) Green peach aphid. A minimum of 90% control, based on pre- and post-emergence treatment and green peach aphid population counts.

(C) Pepper maggot. The test product should demonstrate efficacy equal to or better than products of known efficacy used as reference standards, based on examination of fruit at harvest to determine the percent fruit infested with pepper maggots, pepper coupled with yield data indicating the loss due to pepper maggot.

(viii) Sweet corn. (A) Corn earworm. A maximum of 5% damaged ears for fresh market corn and 25% damaged ears for all other corn.

(B) Fall armyworm. The product should demonstrate efficacy equal to or better than other products of known efficacy used as reference standards, based on post-treatment counts of the fall armyworm and fall armyworm injury.

(C) Corn flea beetle. A minimum of 95% control, based on number of the corn flea beetle per specified number of sweet corn plants per plot, the incidence of bacterial wilt coupled with yield data (noting numbers, weight, size, and general conformation of the ears) from examinations at appropriate intervals.

(D) European corn borer. A minimum of 95% control, based on post-treatment counts at appropriate intervals of the European corn borer and their injury (including the number of larvae, total number of tunnels, and number of infested plants).

(ix) Poled tomatoes: tomato fruitworm, tomato pinworm. A minimum of 90% control, based on examinations at appropriate intervals to determine percent damaged fruit. Extreme care should be exercised to differentiate between tomato fruitworm and tomato pinworm damage.

(x) Tomatoes. (A) Tomato fruitworm. A minimum of 90% control. Refer to poled tomatoes, paragraph (b)(5)(ix) of this section.

(B) Colorado potato beetle. A minimum of 95% control in direct-seeded tomatoes. A minimum of 80% control for summer applications.

(C) Potato aphid. A minimum of 90% control, based on pre- and post-treatment potato aphid population counts and number of injured plants.

(6) Vegetables (greenhouse). (i) Tomatoes, lettuce, cucumber, radish, watercress, endive. (A) Aphids. A minimum of 90% control, based on pre- and post-treatment aphid population counts.

(B) Leaf-eating beetles. A minimum of 90% control, based on pre- and post-treatment beetle population counts.

(C) Cutworms. A minimum of 90% control, based on post-treatment counts of dead larvae found in soil depressions around the bases of injured plants, and percent cutworm-injured plants.

(D) Garden symphylan. A minimum of 90% control, based on pre- and post-treatment population counts at appropriate intervals, of living garden symphylans, as determined from soil samples including part of the root zone of living plants.

(E) Greenhouse whitefly. A minimum of 90% control, based on pre- and post-treatment population counts and leaf examinations to determine percent mortality of immature and adult stages.

(F) Leafminers. A minimum of 90% control, based on pre- and post-treatment examinations of leaves from treated and untreated plants to determine cessation of feeding, shriveling or killed larvae, and examination of mines, correlated to indicate percent dead or living larvae.

(G) Leaf-eating caterpillars. A minimum of 90% control, based on pre- and post-treatment insect population counts and plant injury ratings.

(H) Slugs and snails. A minimum of 90% control, based on post-treatment counts of living and dead slugs in treated areas. Correlate with number of damaged fruit and foliage injury pre- and post-treatment to determine percent control.

(I) Spider mites. A minimum of 90% control, based on pre- and post-treatment mite population counts.

§ 95-3 General soil treatments.

This section is concerned with efficacy testing of invertebrate control pesticides used as soil applications for control of major insect pests of corn sugar beets, peanuts, tobacco, and vegetables as well as a separate discussion on control of imported fire ant (See § 95-3(c)).

(a) General considerations. The testing of pesticide products for use on food crops in home gardens should follow the same procedures as are appropriate for evaluating their commercial use.

(1) Site selection. Crop plantings should be selected which are representative of cultivars (varieties), ages of plants cultural practices, and pesticide populations commonly encountered. When selecting a test site, the soil texture, soil composition, and geographic location must be considered. For general considerations, refer to § 90-2 (a)(3)(iv), (b)(1) through (3).

(2) Plot size. Plots of size suitable for commercial applications should be used. These should have dimensions large enough to avoid any effects

from movement into untreated control and sampling areas. Conditions may dictate that large plots should not be replicated in one planting site which may necessitate additional tests in different plantings.

(3) Number of trials. A minimum of 5 large-scale, geographically-separated trials are generally needed, but the number of trials can vary somewhat due to the accessibility of infestations, fluctuations in pest population pressures, behavior, and other important considerations in the biology of the target pest.

(4) Application techniques and equipment. In-furrow, broadcast, or band applications should be evaluated if these methods are specified on the label. For general considerations, refer to § 90-2 (a)(3)(ii).

(5) Evaluation and reporting procedures. The procedures used to evaluate product performance should be specified. Reports should include yield information, insect counts, percent damage, or other measures for determining the effectiveness of the test product. Results should include the average infestation reduction throughout the sampled post-treatment period compared with that in control plots or in pre- and post-treatment periods. Raw data on pest counts, injury counts, or other measures of control should be summarized. Any adverse effects such as phytotoxicity should be reported. For general considerations, refer to § 90-2 (c)(1) and (3).

(b) Suggested performance standards. Unless otherwise specified, these standards are presented on the basis of pre- and post-treatment pest population counts from the treated as well as the untreated lots and standard comparative treatments. All percentages of control refer to the performance of the test substance (as determined by insect counts, yield data and other measures correlated to insect population pressures) against the vulnerable stage(s) of the target pest, when evaluated according to a recognized treatment program under actual field conditions.

(1) Field crops. (i) Corn. (A) Northern corn rootworm, southern corn rootworm, western corn rootworm. The test substance should compare favorably with other products of known efficacy used as reference standards, when such comparisions are based upon both larval mortality and root damage evaluations. It should show no adverse effects on yield. Use of a root rating scale ranging from 1 (no damage) to 6 (severe damage), a rating no greater that 3 is acceptable.

(B) Cutworms. A minimum of 85% control. In terms of retention, 85% of the stand must be retained.

(ii) Peanuts. (A) Southern corn rootworm. A minimum of 90% control, evaluated by examining random samples of pegs and pods at harvest, and determining southern corn rootworm injury.

(B) Lesser cornstalk borer. A minimum of 90% control, based on pre- and post-treatment lesser cornstalk borer counts.

(C) Granulate cutworm. A minimum of 90% control, based on pre- and post-treatment living and dead larval population counts.

(D) Burrowing bug. A minimum of 90% control, based on population counts of adults and nymphs, obtained from soil samples taken at 10-14 day intervals after insecticide application, and from burrowing bug-damaged kernels at harvest.

(E) Potato leafhopper. A minimum of 90% control, based on pre-treatment and post-treatment adult and nymph potato leafhopper population counts, and counts of the number of leaves with "hopperburn." Post-treatment counts should be taken 10 to 15 weeks after treatment.

(iii) Sugar beets: sugarbeet root maggot. A minimum of 90% control, based on damage ratings ranging from 1 (no damage) to 5 (severe damage). The product must demonstrate a rating less than 2 (light damage) to be acceptable.

(iv) Tobacco. (A) Tobacco wireworm. A minimum of 90% control, based on percent tobacco wireworm damaged plants 10-14 days after transplanting, and root and stem damage within 4 weeks of transplanting.

(B) Darksided cutworm. A minimum of 90% control, based on percent reduction of larval population from counts made as weekly intervals starting one week after transplanting, and continuing until larvae pupate.

(C) Green June beetle. A minimum of 90% control, based on counts of dead larvae on the soil surface approximately 6 weeks after seed germination, when insecticides are applied just prior to seeding. For spring treatments when larvae are active, count dead larvae 2 days post-treatment and continue to determine mortaltiy for 1 week. Two weeks after treatment excavate the treated area to a depth of 6 to 12 inches (15.2 to 30.5 cm) and count all living and dead larvae.

(D) Tobacco flea beetle. A minimum of 90% control, based on pre- and post-treatment tobacco flea beetle population counts, and feeding punctures on damaged leaves throughout the season.

(2) Vegetable crops (field grown). (i) Cabbage, cauliflower, broccoli, brussels sprouts, rutabaga, turnip, onion, beans, peas, potatoes. For the following insect pests of vegetables, the data should indicate that a product demonstrates effective control which is equal to or better than other similar products of known efficacy used as reference standards.

(A) Cabbage maggot (cabbage, cauliflower, broccoli, brussels sprouts). Control is determined from post-treatment counts of cabbage maggot-damaged roots. The actual numbers of cabbage maggot tunnels or infested plants are counted throughout the test period.

(B) Turnip maggot (rutabaga and turnips). Control is determined from post-treatment counts of turnip maggot tunnels are counted throughout the test period.

(C) Onion maggot (onion). Control is determined throughout the test period by counting and removing injured plants until plant mortality cases, and computing treatment effectiveness from the total plant stand.

(D) Seedcorn maggot (beans and peas). Control is determined by counting seedcorn maggot-injured plants per given area, and computing treatment effectiveness from the total plant stand.

(E) Tobacco wireworm (potatoes). Control is determined by counting the number of tobacco wireworm-injured tubers, coupled with yield data at harvest.

(F) Southern potato wireworm (potatoes). Control is determined by counting the number of southern potato wireworm-injured tubers, coupled with yield data at harvest.

(G) Great Basin wireworm and Pacific Coast wireworm on potatoes. Control is determined by counting the number of Great Basin wireworm and/or Pacific Coast wireworm-injured tubers, coupled with yield data at harvest.

(c) Imported fire ant. (1) Site selection. Sites should be selected from several states within the range of the imported fire ant. Usually, three such sites are necessary to provide information in the data derived.

(2) Plot size. (i) Mound application. In general, use a minimum of 10 mounds per plot. Usually, 3 to 4 plots are treated with the candidate compound and 1 plot is used as a control.

(ii) Broadcast treatments. Plots should be of sufficient size to permit the use of the application equipment specified on the label. Replication may be difficult when very large plots are necessary.

(3) Suggested performance standards. (i) Control claims. A minimum of 90% control based on counts made for a minimum of 30 days for mound applications and 60 days for broadcast treatments. Percent control is based upon the percent reduction in both old and new active mounds in the treatment area as compared to precounts and untreated controls.

(ii) Suppression claims. Broadcast treatments only A minimum of 80% control based on counts made for a minimum of 60 days and based upon the adjusted percentage of reduction of the total number of old and new active mounds in the treatment area and untreated controls. A lower percentage of control may be acceptable if testing was done under adverse conditions, but in no case would less than 70% control be acceptable.

§ 95-4 Lawn and turf treatments.

This section concerns efficacy testing of invertebrate control pesticides applied to lawns and turf for protecting these sites from economic injury by insects and certain other invertebrate pests.

(a) General considerations. The testing of a pesticide product for use on home lawns should follow the same procedures as are appropriate for evaluating its commercial use.

(1) Site selecion. Outdoor application sites should be selected from areas where known infestations exist, and must reflect variations in climate or other environmental conditions, edaphic conditions, and geographic distribution. Report test results from at least four different geographical areas where the pest is found. For general considerations, refer to § 90-1 (b)(1) through (3).

(2) Plot size. Plot sizes should be sufficiently large to accomodate commercial application equipment. They usually range from 10' x 10' to a size suitable for commercial aplications. The plots should have dimensions large enough to avoid any movement of the test products into untreated control or sampling areas. Conditions may dictate that large plots should not be replicated in one planting site which may necessitate additional tests in different plantings.

(3) Number of trials. A minimum of 5 large-scale geographically-separated trials are generally necessary, but the number of trials can vary somewhat due to the accessibility of infestations, fluctuations in pest population pressures, behavior, and other important considerations in the biology of the target pest.

(4) Experimental test design. A suitable experimental test design should be used to reduce variability in the test data. Random observations or pre-treatment counts should be used to determine population distribution and to provide guidance in establishing plot location. Examples of experimental designs generally used are randomized complete block design, complete randomized design, and latin square design.

(5) Application techniques and equipment. Dusts may be applied with a hand duster or lawn fertilizer spreader; granules and dry baits, with a lawn fertilizer spreader; wettable powders and emulsifiable concentrates (after dilution with water) with a compressed air or knapsack sprayer, a hose proportioner or a watering can. For general considerations, refer to § 90-2 (a)(3)(i).

(6) Evaluation and reporting procedures. The procedures used to evaluate product performance should be specified in the presentation of the data Reports must include insect counts, percent damage or other measures for determining the effectiveness of the test product. Results should include the average infestation reduction throughout the sampled post-treatment period compared with that in control plots in pre- and post-treatment periods. Raw data on pest counts, injury counts, or other measures of control should be summarized and submitted. Since grasses or other lawn plants differ significantly in their sensitivity to chemicals, and some high quality lawns and golf greens represent a sizeable monetary investment, it is important to observe any adverse effects such a phytotoxicity which might occur after treatment. For general considerations, refer to §§ 90-2 (c)(1), (2) and (d)(1) through (4).

(b) Suggested performance standards. These standards apply to control of insect pests on all types of lawn turf grasses. Unless otherwise specified, these standards are presented on the basis of pre- and post-treatment pest population counts from the treated as well as the untreated plots and standard comparative treatments. All percentages of control refer to performance of the test substance (as determined by insect counts, yield data and other measures correlated to insect population pressures) against the vulnerable stage(s) of the target pest, when evaluated according to a recognized treatment program under actual field conditions. For (4), (5), (6), and (7) (below), the poduct should demonstrate effective control which is equal to or better than other similar products of known efficacy used as reference standards.

(1) Chinch bugs. A minimum of 95% control, expressed as knockdown ability of the pesticide when counts are taken within 1 week of the data of application. A minimum of 90% control is needed for additional residual claims of greater than the 1 week period.

(2) White grubs. A minimum of 90% control based upon population reductions of the most damaging larval instars when expressed as a factor of the numbers of white grubs per unit area (sq. ft., sq. meter, etc.) and compared to untreated controls.

(3) Sod webworms. A minimum of 90% control based on population reduction counts obtained by counting larvae on a specified area (e.g., a 0.6m x 0.6m section of each plot) that emerge in 10 minutes as a result of sprinkling 0.02-0.05% pyrethrum on the test area.

(4) Mole crickets. Different methods may be used to determine control. The most common method is to count dead and moribund mole crickets on the turf in each plot. Counts are made for four to seven days after treatment. A second method is to drench a 1 m^2 area with 21% pyrethum and count the mole crickets that emerge within 15 minutes. A third method has been to count surface burrows in open spaces after irrigation or rain.

(5) Spittlebugs. Control is based on the number of spittlebugs determined in a specified size area of each plot; counts are made one, three, and seven days after treatment.

(6) Lepidopterous larvae. Grass loopers of the genus *Mocis* and other turf-feeding Lepidoptera (*Spodoptera* spp.). Based on pre-treatment and post-treatment counts at 0, 24, and 72 hours.

(7) Margarodid scales (ground pearls). Based on counts made from soil core samples taken during pre-treatment and at intervals of up to one year after treatment.

§ 95-5 Outdoor woody ornamental plant treatments.

This section concerns efficacy testing of invertebrate control pesticides used on outdoor woody ornamental plants for protection from economic injury by insects and certain other invertebrate pests. Such plants include a large and constantly increasing number of cultivars, embracing more than 1000 species, 150 genera, and 60 families. The most common of these include arborvitae, azalea, boxwood, camellia, chamaecyparis, cotoneaster, crabapple, dogwood, euonymus, forsythia, holly, honey locust, juniper, laurel, lilac, magnolia, oleander, palm, privet, pyracantha, redbud, rhododendron, spirea, viburnum, and yew.

(a) General considerations. The testing of a pesticide product for use on woody ornamentals and shrubs in home gardens must follow the same procedures as are appropriate for evaluating its commercial use.

(1) Site selection. Plantings for experimentation should be selected which are representative of cultivars (varieties), ages of plants, cultural practices, and pest populations commonly encountered. When selecting a test site, other factors such as climate, soil texture, other substrates, and geographic distribution must be considered. For general considerations, refer to § 90-2 (a)(3)(iv), (b)(1) through (3).

(2) Plot size. Plot size should be sufficiently large to accomodate commercial application equipment. Plots should be sufficiently separated so that there is essentially no opportunity for drift on untreated controls and sampling areas. Conditions may dictate that large plots not be replicated in one planting; therefore, additionals test may be needed at different locations.

(3) Number of trials. A minimum of 3 large-scale, geographically-separated trials are generally necessary, but the number of trials can vary somewhat due to the accessibility of infestations, fluctuations in pest population pressures, behavior, and other important considerations in the biology of the target pest.

(4) Application techniques and equipment. Application techniques should be appropriate for the use contemplated and the size of the test plots. Compressed air sprayers are frequently used and results are comparable to those obtained from power equipment, provided that the same dilution is used and the same amount of toxicant is applied to each plant. Other application techniques such as aerial or ultra-low volume should be evaluated if these methods are specified on the label. For general considerations, refer to § 90-2 (a)(3)(i), (ii).

(5) Evaluation and reporting procedures. The procedures used to evaluate product performance data should be specified. Reports should include yield information, insect counts, percent damage, or other measures of determining the effectiveness of the test products. Results should include the average infestation reduction throughout the sampled post-treatment period compared with that in control plots or in pre- and post-treatment periods. Data on pest counts, injury counts, or other measures of control should be summarized. Due to the complex nature of pesticide usage on woody ornamentals and the sizable number of host plants involved, it is necessary that plant tolerance to such applications be determined. This is an integral part of effectiveness testing of pesticide products since plants differ in their responses to chemicals, and a product registered for use against a pest on one host may be registered for use against the same pest on another host, provided phytotoxicity data indicates plant tolerance. For general considerations, refer to § 90-2 (c)(1), (2), and (d)(1) through (4).

(b) Suggested performance standards. These standards apply to control of insect pests on outdoor woody ornamental plants. Unless otherwise specified, these standards are presented on the basis of pre- and post-treatment pest population counts from the treated as well as the untreated plots and standard comparative treatments. All percentages of control refer to the performance of the test product (as determined by insect counts, yield and/or damage data and other measures correlated to insect population pressures) against the vulnerable stage(s) of the target pest, when evaluated according to a recognized treatment program under actual field conditions.

(1) Adelgids. A minimum of 90% control, when measured as a factor of a reduction in new galls as compared to pre-treatment and post-treatment counts.

(2) Aphids. A minimum of 90% control based upon counts of living aphids made during pre-treatment and at least 48 hours during post-treatment, considered as adjusted mortality when compared to water-treated controls.

(3) Wood boring insects. Almost complete (99%) control in specimen plantings and 85% control in production plantings, when based upon a measurement of adult emergence and frass counts, as appropriate.

(4) Bud destroying insects and tip destroying insects. A minimum of 90% control, evaluated as a factor of insect counts, adult emergence, or pupal counts and correlated to appropriate damage indices.

(5) True bugs. A minimum of 90% control, as based upon adjusted mortality and correlated to damage reduction.

(6) Foliage feeding insects (defoliators). A minimum of 90% control, based upon pre- and post-treatment adjusted mortality correlated to damage reduction.

(7) Eriophyid mites. A minimum of 90% control, based upon a reduction in new galls or damaged plant parts.

(8) Gall wasps and gall aphids. A minimum of 90% control, when measured as a factor of a reduction in new galls, or the percentage of galls containing living insects.

(9) Leafminers. A minimum of 90% control, based upon the numbers of damaged leaves on treated and untreated plants coupled with demonstration of the ability of the material to inhibit the development of new mines on previously infested leaves.

(10) Mealybugs. A minimum of 90% control, based upon population reductions and correlated to a reduction of damage.

(11) Root feeding insects. A minimum of 90% control, based upon population reductions and adjusted mortalities as compared to damage reduction.

(12) Scale insects. For scale crawlers, a minimum of 90% control is required when applications are compared to water-treated controls. For scale adults, a minimum of 85% control is required based upon adjusted mortality as compared to untreated controls and relative plant vigor.

(13) Tetranychid mites. A minimum of 90% control, based upon population reduction as compared to untreated check plots, when counts are made with a mite-brushing machine, ketone extraction, or other accepted sampling method.

(14) Thrips. A minimum of 90% control, based upon population reductions as compared to untreated samples.

§ 95-6 Greenhouse floricultural treatments.

This section is concerned with efficacy testing of invertebrate control pesticides used on greenhouse floricultural crops for protection from economic injury by insects and certain other invertebrate pests such as aphids, beetles, cutworms, garden symphylan, greenhouse whitefly, leafminers, leaf-eating caterpillars, slugs, snails, and spider mites.

(a) General considerations. (1) Site selection. Uses of insecticide products in greenhouses are similar, regardless of location, due to controlled environments. Data from several individual greenhouses are adequate as long as such locations cover the range of variations in temperature, relative humidity, soil or other substrates, or growing practices likely to be encountered. For general considerations, refer to § 90-2 (b)(1), and (2).

(2) Plot size. Plots size should be sufficiently large to accomodate commercial application equipment. Conditions may dictate that large plots not be replicated in one planting. In this event, additional tests may be necessary at different plantings

(3) Number of trials. At least 5 individual trials, divided among 3 locations, each utilizing a single greenhouse, will provide adequate data.

(4) Application techniques and equipment. Ultra-low volume spray applications should be evaluated if these methods are specified on the label. For general considerations, refer to § 90-2 (a)(3)(ii).

(5) Evaluation and reporting procedures. The procedures used to evaluate product performance data should be specified. Reports should include yield information, insect counts, percent crop damage, ot other detrimental effects (also see Subdivision J), or other measures of determining the effectiveness of the test product. Results should include the average infestation reduction throughout the sampled post-treatment period compared with that in control flat or in pre- and post-treatment periods. Data on pest counts, amount of injury, or other measures of control should be summarized. For general considerations, refer to §§ 90-2 (c)(1), (2), and (d)(1) through (4).

(b) Suggested performance standards. These suggested standards apply to control of insect pests on greenhouse floricultural crops. Unless otherwise, specified, these standards are presented on the basis of pre- and post-treatment pest population counts from the treated as well as the untreated plots and standard comparative treatments. All percentages of control refer to the performance of the test substance (as determined by insect counts, yield data, and other measures correlated to insect population pressures) aginst the vulnerable stage(s) of the target pest, when evaluated according to a recognized treatment program under actual use conditions.

(1) Twospotted spider mite. A minimum of 95% control, expressed as population reductions from samples taken from leaves or flowers, as appropriate.

(2) Aphids. A minimum of 99% control based upon population reductions in both foliar and floral plant parts, as appropriate, or based upon the percentage of infested plants or flowers; or a minimum of 90% control when expressed as adjusted mortality based upon water-treated (water spray only) controls.

(3) Flower thrips. Almost complete (99%) control of exposed flower thrips, of 90% control of all flower thrips, as sampled by the wash method, mechanical method, or irritation method, and compared to untreated controls.

(4) Iris borer. A minimum of 85% control, based upon a reduction of infested rhizomes as derived from dissecting sample material from both treated and untreated plots.

(5) Mealybugs Almost complete (99%) control, based upon comparison with untreated checks reported as the mean number of individuals per plant.

(6) Greenhouse whitefly. Almost complete (99%) control of the adult stage, based upon comparison with untreated checks: or for immature stages, a minimum of 95% control of either larvae or "puparia" when measured as a factor of pre- and post-treatment counts of living individuals in the presence of acceptable populations recorded in untreated checks.

§ 95-7 Shade trees and forest land treatments.

This section is concerned with efficacy testing of invertebrate control pesticides used on shade trees and forest lands for protection against direct and indirect economic injury by insects and certain other invertebrate pests. Forest pest control or suppression programs are usually conducted under the supervision of state or Federal agencies. These programs are generally directed at a single species of pest often of regional significance. As such, extensive areas are often treated. The treatment of extensive areas requires the careful development of data concerning effects on non-target organisms. Data on directions for use also should be obtained since these treatments usually involve application procedures which are different from typical agricultural techniques. The objectives of the program may be for suppression or management of a vector of forest diseases, for protection of foliage, or control of a forest insect.

(a) Definitions. The following definitions apply to this section:

(1) "Foliage protection" is defined as that level of pest population reduction necessary to preserve the tree foliage to the point that mortality

of the tree(s) does not occur.

(2) "Control" is defined as that level of pest poplulation reduction necessary to preclude the need to treat the succeeding generation of the pest.

(b) General considerations. (1) Site selection. Experimental plots must be located in areas where a readily measurable population of pest insect exists, where a predominance of preferred host sites (trees) suitable for population sampling are present, and where the insect pest population is in a healthy condition. For general considerations, refer to § 90-2 (a)(3)(iv). The insects evaluated must be minimally affected by natural epizootic factors. For defoliating insects, plots should be located within pest populations that are increasing in severity and with no more than one year's noticeable defoliation of damage prior to the test season. Plots are to be located in geographic areas representative of the distribution of the pest. For general considerations, refer to § 90-2 (b)(3).

(2) Plot size. Plot size should be adequate to evaluate the proposed application techniques. Minimum plot size is generally 50 acres (20.3 hectares) for small single-engine fixed-wing aircraft and 20 (8.1 hectares) acres for helicopter application. The acreage necessary to evaluate products intended for large scale control programs utilizing larger aircraft may increase to several hundred acres (hectares) per plot depending on the equipment used.

(3) Number of trials. A minimum of 3 large-scale, geographically-separated trials are generally necessary, but the number of trials can vary somewhat due to the accessibility of infestations, fluctuations in pest population pressures, behavior, and other important considerations in the biology of the target pest.

(4) Application techniques and equipment. Data should be developed from large scale plots utilizing application equipment and techniques that can be expected to be used in control programs. If the product is likely to be applied by multi-engine aircraft, then test results from experiments using multi-engine aircraft should be developed. Forest applications generally involve low volume or ultra-low volume spray rates because of economics and logistics. The problem of delivery of this small amount of pesticide to a target site becomes paramount. Information on droplet size should be reported for each forest pesticide . For general considerations, refer to § 90-2 (a)(3).

(5) Evaluation and reporting considerations. Depending upon the objectives of the program and proposed pesticide usage, product evaluation may involve sampling of the pest poplulation or determination of the level of defoliation. Sampling may involve pre- and post-treatment determination population levels, comparison with an untreated control plot, or with levels of known economic damage. The development of insect life histories in relation to effects upon host tree mortality of injury is encouraged. Where the objective of the program is primarily foliage protection, methods for estimating the loss of foliage must be employed. Each method should also be accompanied by data substantiating that the defoliation is attributable to the specified pest under evaluation. The use of infrared aerial photography can be use to great advantage in determining degree of defoliation

over extensive areas. Potential nontarget effects include not only hazards to wildlife and aquatic organisms (Subdivision E), but also effects such as potential damage to automobile finishes and similar structures subjected to pesticide deposition, and phytotoxic effects upon desirable trees and understory shrubs. An evaluation of any non-target effects should be submitted or referenced with each application for registration. For general considerations, refer to § 90-3 (f)(4)(ii).

(6) Untreated controls and comparative treatments. The proposed product should be tested against a registered comparative treatment applied to an adjacent area of planting, or compared to an untreated control plot. Acceptable levels of damage may be used as a comparison, provided they are clearly defined.

(c) Specific considerations. These considerations apply to control of insect pests on shade trees and forest land. Forest applications directed against foliage-feeding insects (defoliators) generally are used for either foliage protection, or control of the pest. Gypsy moth, spruce budworm, and Douglas-fir tussock moth programs are discussed in detail.

(1) Gypsy moth. Generally foliage protection and control are evaluated in programs with the gypsy moth as the target pest. Experimental areas must have a minimum of 200 egg masses per acre (hectare), and preferably not more than 1000. The population should be on the increase, with a minimum of virus incidence. Plots should contain a predominance of preferred host trees (oaks). Pilot plot size should be no smaller than 50 acres (20.3 hectares) and be replicated at least 4 times. Operational tests of at least 300 acres (121.5 hectares) should be included. The relationship of larval development to stage of host leaf expansion should be specified. Usually applications are directed against second instar larvae when the oak foliage is 50 to 75% expanded. The methodology for sampling of the pest population or estimation of defoliation should be given. Foliage protection should be estimated in 20% or smaller increments on all tree species in subplots on both test and control plots. Usually 20 one-fortieth acre (hectare) subplots per plot are adequate. Population reduction or control should be determined by measuring the pre- and post-treatment egg mass counts. Additional evidence of mortality due to treatment must be determined by measuring residual larval populations counted on 24-inch (61.0 cm) terminal branches, by timed counts of larvae, counts on subplots, or larval counts under burlap bands.

(2) Spruce budworm and western spruce budworm. The following conditions apply to pesticide products intended for both the spruce budworm and western spruce budworm. The population density in plots should be of sufficient intensity to cause economic injury in the absence of treatment. This number is usually 15 larvae per 15-inch (38.1 cm) branch taken from mid-crown of the tree. The population should be healthy with minimum indication of natural epizootic factors present. There should be a predominance of the preferred host species (spruce/fir). Plot size should be appropriate for the application of the equipment employed. Since spruce budworm programs usually employ large aircraft, plots of 500 acres (202.5 hectares) or greater are usually necessary. Information on the stage of larval development in relationship to tree development should be provided. Most spruce budworm programs are directed towards the late fourth instar larvae at the time of bud break of the host tree. The sampling method should be described in detail. Adequate sampling for both spruce budworms involves the collection

of branch samples from the mid-crown of 30- to 50-foot-high (9.1- to 15.2-meter) trees. At least 15 different trees should be randomly selected per plot with two to four 15-inch (38.1 cm) branches taken per tree. Counts should be expressed as the number of larvae per 15-inch (38.1 cm) branch or number per 100 shoots or buds. The degree of tree top-kill and amount of regrowth after treatment should also be given.

(3) Douglas-fir tussock moth. Population density within plots should be of sufficient intensity to cause economic injury in the absence of treatment. This level is 20 or more egg masses per 1000 square inches of foliage. The larval population should be essentially free of the influence of virus infection. Preferred host trees (Douglas-fir or True firs) should predominate in the experimental plots. Plot size should be adequate for the type of application equipment employed. Plots should be 500 acres (202.5 hectares) or larger. Information on the timing of application should be developed. Application timing in relation to percent egg hatch and the details of sampling methodology should be provided. Sampling must involve counts of the number of larvae per 18-inch (45.7 cm) branch taken from the mid-crown of 30- to 50-foot-high (9.1- to 15.2-meter) trees. A minimum of 15 trees must be randomly selected per plot. The population level should be expressed as the number of larvae per 1000 square inches of foliage or number per 18-inch (45.7 cm) branch. Foliage protection should be measured by optical examination of the volume of feeding per 100 buds per tree expressed as percent defoliation in increments of 10%.

(4) Other pest species. Specific data considerations and test standards have not been developed for forest insect pests other than those listed above. Registration guidance for other pests will be handled on a case-by-case basis. Registrants are urged to contact the Agency as early in the experimental program as possible to develop data needs.

(d) Suggested performance standards. (1) Gypsy moth. Products will be acceptable for foliage protection if 50% of the foliage is preserved as a result of larval feeding reduction due to the treatment. Products will be acceptable for control of the gypsy moth larvae if reduction of the population to less than 2000 egg masses per acre (hectare) is achieved as a result of the treatment, compared to significant population increases in untreated plots.

(2) Spruce budworm and western spruce budworm. Products will be acceptable for foliage protection if the results of treatment show protection of 50% of the current year's foliage. Products demonstrating control must reduce the population to levels where retreatment in succeeding seasons will not be necessary.

(3) Douglas-fir tussock moth. Products will be considered acceptable for foliage protection if the results of treatment show protection of 75% of the current year's foliage. Products will be acceptable for control of Douglas-fir tussock moth larvae if reduction of the succeeding year's population is reduced to less than 20 eggs (0.1 egg masses) per 1000 square inches of foliage as a result of the treatment compared to significant population increases in untreated plots.

§ 95-8 Livestock, poultry, fur, and wool-bearing animal treatments.

This section concerns efficacy testing of invertebrate control pesticides used on cattle, horses, sheep, goats, swine, chickens, turkeys, other domestic fowls, and fur-bearing animals such as mink and rabbits, for control of the major arthropod pests that parasitize these animals.

(a) General considerations. (1) Site selection. Pests of livestock, poultry, fur, and wool-bearing animals include, but are not limited to, cattle grubs, nose bot, sheep ked, various biting flies (horn fly and stable fly), lice, ticks, housefly, face fly, mosquitoes, and sarcoptic mange mites. Evaluating an insecticide that is to be applied directly to such animals to control these pests should generally be based on adequate tests on representative animals from herds or flocks in at least 5 widely-separated regions where the insecticide is to be marketed, unless applicability so limits use of the pesticide in special representative regions. Tests on dairy cattle may not be necessary if comparable to efficacy data are reported from tests using beef cattle. For general considerations, refer to § 90-3(b)(3).

(2) Sample size. Sample size should be representative of the number of animals in a particular treatment group, which can range from one to a herd or flock of thousands.

(3) Number of trials. A minimum of 5 large-scale geographically-separated trials are generally necessary, but the number of trials can vary somewhat due to the accessibility of infestations, fluctuations in pest population pressures, behavior, and other important considerations in the biology of the target pest.

(4) Dosage selection. Dosage levels and concentrations should be identified in the laboratory or small-scale tests before field testing. It is usually necessary to repeat an application only once or twice for lice, ticks and sarcoptic mange mites. The data should include support of the schedule of repeated applications normally required against flies and their continuous population pressure. For general considerations, refer to § 90-2 (a)(2).

(5) Application techniques and equipment. (i) Single oral dose. For insecticides administered orally as drenches, boluses, or in capsules, care should be taken that animals receive the entire dose. Record the formulation, final concentration of active ingredient, total amount administered, and dosage in terms of mg of active ingredient per kg of body weight of animal. The regulation of many internal applications comes under the authority of the Food and Drug Administration, Bureau of Veterinary Medicine. Some of these pesticides may fall into this category.

(ii) Feed treatment. Insecticides administered to animals as part of a feeding regimen should be mixed into the entire feed ration or fed in a small amount of feed which, when consumed, is followed by untreated feed. Record the formulation, final percent content of active ingredient (ppm in feed), or total mg of active ingredient per kg body weight of animal, amount of feed or treatment consumed, and length of treatment period.

(iii) Water treatment. Some insecticides may be administered to animals through drinking water. Animals may be treated individually or in groups, which, when having consumed the treated water, are given untreated water. Regardless of the size of the group, the following should be recorded: the formulation, final concentration of active ingredient in terms of ppm in water or mg of active ingredient per kg of body weight of animal, average consumption of water per animal, and duration of treatment.

(iv) Mineral, salt, or protein supplement. Insecticides of this type are generally formulated at low concentrations in mineral, salt, or protein supplements are offered free choice to animals. Because consumption of salt, mineral, and protein supplement varies considerably from animal to animal, it is important to determine whether or not all animals consumed treated materials. Record the formulation, final concentration of active ingredient in terms of percentage or ppm of treated supplement, average consumption per animal per day, dosage in terms of mg active ingredient per kg body weight per day, and length of treatment period.

(v) Injections. For insecticides given to animals in the form of intramuscular, intraperitoneal, or subcutaneous injections, record the formulation, amount of material injected per animal, location of injection, and dosage in terms of mg of active ingredient per kg of body weight.

(vi) Whole-body sprays. For insecticides applied to animals as whole-body sprays, care should be taken that animals are treated thoroughly and that enough pressure is used to penetrate hair coat and assure wetting of the skin. A variation of the whole-body spray is the use of spray-dip machine to apply spray to animals. With either method, record the formulation, final concentration of active ingredient, equipment used and application techniques, and average volume of spray applied per animal.

(vii) Dip. For insecticides used to charge dipping vats, animals should be immersed thoroughly in the dip fluids. Record the formulation, final concentration of active ingredient, volume of liquid in the vat, age of charge at time of dipping, number of animals dipped, and data on recharging (if necessary). Chemical analyses of active ingredient in vat fluids before and after dipping are necessary to determine the actual amount of active ingredient in the vat fluid.

(viii) Pour-on treatment. Insecticides applied to animals by the pour-on technique, ready-to-use formulations, or emulsifiable concentrates diluted with water or oil, are poured down the backline of animals in ounce (milliliter) rates. In an extension of this technique, ready-to-use formulations are applied to a spot on the backline at milliliter rates. Record formulation, diluent, final concentration of active ingredient, amount applied per animal, area treated, and dosage based on mg active ingredient per kg body weight of animal.

(ix) Dust treatment (livestock). Insecticides are applied as dusts by power duster or by hand or contained in dust bags and placed in the pasture for free-choice use or placed in openings to feed, mineral, and/or water sources so that animals are forced to use them on a daily basis. With cattle grub control, it is important that dust bags be located so that animals are forced to treat themselves on a daily basis to insure that sufficient insecticide is applied for cattle grub control. In tests with dust bags for control of ectoparasites, such daily treatment is not essential. Record formulation, final concentration of active ingredient, average amount of dust per animal, location of dust bags, and length of treatment period.

(x) Back spray. In tests to control cattle grubs, the animal's backs are sprayed thoroughly with the contact insecticide. Care is taken to force insecticide into the warble openings in the animal's backs. Record formulation, final concentration of active ingredient, equipment used, application techniques, and average volume of spray applied per back.

(xi) Floor or litter treatment. Insecticides are applied as mists or fogs, dust, or granules directly to floor areas, litter, or nest areas. Record formulation, final concentration of active ingredient, equipment used, amount of insecticide per square meter of surface treated, and total surface area treated.

(xii) Dust box treatment (poultry). Insecticides formulated as dusts or granules are placed into dust box containers and fowl allowed to treat themselves. Record formulation, diluent, final concentration of active ingredient, amount of material per dust box, length of treatment period, number of birds, and average amount of material used per bird during treatment period.

(xiii) Vapor treatment. Strands, cords, or other devices impregnated with insecticides are attached underneath or around cages containing infested birds. Insecticides volatilize from the impregnated surfaces and kill ectoparasites on birds. Record the formulation, impregnated material, final concentration of active ingredient, length or weight of impregnated material per bird or per cage containing a specific number of birds, and length of treatment period. Low volume or ultra low volume application must be evaluated if these methods are to be specified on the label. For general considerations, refer to § 90-3 (a) and (b).

(6) Record of toxicity and other adverse effects. Adjusted average daily gains (ADG) on the test and control groups should be recorded and reported. In testing petroleum oil formulations, the possible toxic effects on the animal from the petroleum oil must be evaluated. For example, small amounts of certain solvents cause tolerable minor itching and burning for short periods after application. Higher concentrations may cause death if not diluted before application. The recommended method of treatment for pour-on and other ready-to-use products containing oils should be tested to ensure that the recommended method of treatment will not result in excessive dosages of oil which may evoke adverse reactions in treated animals. The final use dilution of emulsifiable concentrations should be tested to ensure that it does not contain dangerous amounts of oil. Insecticides which are tested for use on livestock, poultry, of fur or wool-bearing animals should not injure the animal even when application is repeated over a long period of time; the margin of safety to the treated animal is a vital consideration

in determining usefulness. Neither should the insecticides appear illegally in or on meat, meat by-products, milk, or eggs. If the treated animal is less than 3 months of age (excluding poultry), the effects of stress caused by a particular operation such as castration, dehorning, or other similar procedures, should be evaluated in conjunction with toxicity data. This is especially important for feed-through insecticides. For general considerations, refer to §§ 90-3 (d)(1) through (4), and for other requirements on toxicity, refer to Subdivision F.

(7) Evaluation and reporting procedures. The evaluation procedures used should be specified in the presentation of the data. Reports should include formulations, final concentrations of active ingredient in terms of ppm in water or percent active ingredient in dusts, dosage in terms of mg of active ingredient per kg of body weight of animal, length of treatment period, equipment used, and other similar factors. The average daily gain in weight should be reported for any tests of 7 to 14 days duration.

(8) Untreated controls and comparative treatments. When it is impractical to maintain untreated control animals, the effectiveness of the test product may be measured by comparative treatments with products of well-known efficacy used as reference standards. To evaluate the effect of treatments, records should be kept on the comparative changes in meat, milk, or egg production or some similar measurement; and insect counts following each treatment. The number of animals in the control or comparative treatment groups must be equal to the numbers of animals in a treatment group in small scale tests; in large-scale tests, only a small portion of the animals need to be untreated or given a standard treatment. Control groups should contain animals of the same general size, age, condition, and origin as those in the treatment groups and have similar infestations of parasites or be exposed to similar populations of arthropod parasites.

(b) Suggested performance standards. Unless otherwise specified, these standards are presented on the basis of pest population counts from treated compared to untreated animals. All percentages of control refer to the performance of the test product (as determined by insect counts, yield of meat and milk, and any other measures correlated to insect population pressures) against the vulnerable stage(s) of the target pest, when evaluated according to a recognized treatment program under actual field conditions.

(1) Cattle (beef and dairy). (i) Cattle grubs. All of the following percentages are based upon the reductions of larvae (warbles) in the backs of animals, as derived from monthly observation and compared to untreated control groups.

(A) Systemic treatments: single oral dose, feed treatment, drinking water treatment, mineral salt or protein supplements, injections, whole-body sprays, dips, pour-ons. A minimum of 90% reduction in infestation.

(B) Contact treatments: dusts and sprays. A minimum of 75% reduction in infestation.

(ii) Horn fly. The percentages of control for the horn fly are based upon pre- and post-treatment counts coupled with a comparison to separate untreated control groups. Such percentages are derived from actual counts of the number of adult flies per side per animal, and may additionally be correlated average daily weight or milk production.

(A) Feed treatment, drinking-water treatment, and mineral salt or protein supplements. A minimum of 90% control of emerging adults along with a 70% reduction of adults on the cattle as measured by side or whole-body counts.

(B) Whole-body sprays, dips, pour-ons. A minimum of 90% reduction in infestation for 3 weeks.

(C) Dusts. A minimum of 95% reduction in infestation for 2 weeks.

(D) Backrubbers. A mimimum of 90% reduction in infestation, under continued use for one month.

(E) LV, ULV and waxed-bar applications. A minimum of 90% reduction in infestation under continued use.

(iii) Other biting flies: (stable fly, deer flies, and horse flies). The percentages of control given for these pests are based upon the feeding rates of flies before treatment compared to the feeding rates of flies on the same cattle after treatment as correlated to the feeding rates on untreated animals taken at the same time. Such feeding rates should be expressed as populations of each separate pest species observed consistently at a particular time of day associated with maximum fly activity.

(A) Whole-body sprays, dips, pour-on, and dusts. A minimum of 90% reduction in infestation one day after application and 75% reduction in infestation one week after application.

(B) Backrubber, LV, and ULV applications A minimum of 90% reduction in infestation under continued use.

(iv) Face fly: whole-body sprays, dips, pour-ons, dusts, backrubbers, LV, ULV, applications, smears, baits, ointments, and face wipes. Reduction in infestation should range from 20-60% for 2-3 days from a single application, based upon a comparison of the numbers of flies per animal pre- and post-treatment correlated with populations of the pest occurring on adjacent untreated herds observed at the same time.

(v) Lice. The percentage of control for lice are expressed as factors of population reductions derived from skin examinations on those areas of the body most susceptible to the particular species of louse. Such percentages are additionally based upon pre- and post-treatment population counts of both treated and untreated animals.

(A) Whole-body sprays, dips, and pour-ons. A mimimum of 90% reduction in infestation for one month after treatment

(B) Dusts. A mimimum of 90% reduction in infestation for a single application for one month after treatment.

(C) Back rubbers, LV, and ULV applications. A minimum of 90% reduction in infestation under continued use.

(vi) Ticks. The percentage of control listed as suggested performance standards for ticks are based upon pre- and post-treatment counts on the same animals or as the average number counted on untreated controls during the same post-treatment intervals. If pre- and post-treatment counts are utilized as a basis for computing the percentage, then untreated control counts should still be reported to permit correlation with natural tick population dynamics for the site/pest complex.

(A) Whole-body sprays, dips, pour-ons, and dusts. A mimimum of 90% reduction in infestation one day after application and 75% reduction in infestation for one week after application.

(B) Back rubber, LV, and ULV applications. A minimum of 90% reduction in infestation under continued use.

(vii) Scab mite and various mange mites: dips and whole-body sprays. Performances of candidate materials for scab and mange mite [1] control is based upon a determination of pre- and post-treatment population counts obtained from skin scrapings and subsequent microscopic examination. Such treatments should be compared to control groups in separate areas. The standard is complete control from two treatments at two week intervals, or as the product is used according to label directions.

(viii) Screwworm: dips, whole-body sprays, dusts, and smears. All larvae in pre-infested wounds must be killed, and reinfestations must be prevented for a two-week period.

(2) Horses. (i) Horse bots: stomach tube, pour-on, intramuscular injection, and feed treatment. A minimum of 90% reduction as determined by counts of expelled larvae compared with counts of those larvae remaining in the animal after treatment (to be determined either by necropsy or by trichlorfon drench).

(ii) Ticks, lice and mange mites [1]. (A) Whole-body sprays, dips. A minimum of 75% reduction in tick infestation at the end of one week, and 90% reduction in louse and mange mite infestations at the end of 2 weeks, based upon pre- and post-treatment counts in comparison with untreated controls.

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[1] The mange mites include Psorergetes mange mites, demodectic mange mites, psoroptic mange mites, and sarcoptic mange mites.

(B) Ear treatment for ticks only. A minimum of 90% reduction in infestation for one week and 75% reduction in infestation for one month, based upon pre- and post-treatment counts in comparison with untreated controls.

(iii) Flies: dips, whole-body sprays, fine-mist sprays, toxicants and/or repellents in aerosols, sponge-ons, wipe-ons, smears, baits, and treated halters. The percentage given as a suggested performance standard for fly control are based upon a reduction in infestation numbers for a minimum period of 3 hours or that period of protection to be claimed on the label, whichever is shorter. A minimum of 90% reduction in infestation under continued use.

(3) Sheep and goats. (i) Sheep lice and goat lice: dips, sprays, pour-ons, dusts, and treated collars. A minimum of 90% reduction in infestation for one month; based upon average numbers of lice on treated and untreated animals at specific intervals after treatment.

(ii) Sheep ked: dips, sprays, pour-ons, dusts, and treated collars. A minimum of 90% reduction in reinfestation for one month, based upon pre- and post-treatment counts as correlated to control populations.

(iii) Scab mite and various mange mites [1]: dips. A minimum of 90% reduction in infestation based upon the number of infested animals post-treatment as compared to the total number of infested animals pre-treatment and correlated to control population fluctuations, as determined pre- and post-treatment skin scrapings.

(iv) Fleeceworms and screwworm: sprays, dips, dusts, and smears. Must initially kill all existing larvae and prevent subsequent reinfestation for a period of 2 weeks.

(v) Sheep bot fly: single oral treatments, feed or water additives, injection, and nasal treatments. A minimum of 90% reduction in infestation following treatment, based upon the pre- and post-treatment infestation rates and the infestation rate of similar untreated animals examined at the same time.

(4) Swine. (i) Hog louse. The suggested performance standards for the hog louse are expressed as percentages based upon the average numbers of lice on the animals, pre- and post-treatment, as compared to the average numbers of the hog louse on untreated swine. This percentage of control should be demonstrated for the period of time claimed on the label unless otherwise stated.

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[1] The mange mites include Psorergetes mange mites, demodectic mange mites, psoroptic mange mites, and sarcoptic mange mites.

(A) Whole-body sprays, dips, dusts, and pour-ons. A minimum of 90% reduction in infestation for one month following treatment.

(B) Back-rubbers and oilers. A minimum of 90% reduction in infestation under continued use.

(C) Litter treatments. A minimum of 90% reduction in infestation for a period of one month after one or two treatments.

(ii) Hog mange mite: dips and sprays. A minimum of 90% reduction in infestation for a period of one month after one or two treatments. Percentages are derived from a comparison between skin scrapings taken both pre- and post-treatment from treated and untreated animals.

(5) Chickens, turkeys, and other domestic fowl. (i) Lice: dips, sprays, mist sprays, dusts, floor or litter treatments, dust box treatments, and vapor treatments. A minimum of 90% reduction in infestation, as determined by comparing the average number of lice per bird in treated groups with the average number of lice per bird in untreated groups, both pre- and post-treatment, for a period of one month.

(ii) Northern fowl mite and tropical fowl mite: dips, sprays, mist sprays, dusts, floor or litter treatments, dust box treatments, and vapor treatments. A minimum of 90% reduction in infestation for a period of one month after treatment, based upon reductions in the number of mites and the relative degree of feather discoloration in the vent area.

(iii) Turkey chigger: dips, mist sprays, dusts, floor or litter treatments, dust box treatments, and vapor treatments. A minimum of 90% reduction in infestation for a period of one month after treatment, based upon the numbers of lesions pre- and post-treatment on both treated and control groups of birds.

(iv) Manure-inhabiting fly larvae. (A) Feed treatment. A minimum of 90% reduction in infestation under continued use, based upon both adult fly reductions determined either by manure emergence or manure bioassay testing, comparing both treated and control groups.

(B) Fly larvicides to manure. A minimum of 90% reductions in infestation for a period of 2 weeks after treatment, based upon the same criteria used for feed treatment in paragraph (b)(6)(iv)(A) of this section.

§ 95-9 Treatments to control pests of humans and pets.

This section is concerned with efficacy testing of invertebrate control pesticides used on humans and pets. Humans may be infested by three types of lice: the body louse, the head louse, and crab louse. The demonstration of effectiveness against any species should therefore be based on tests against lice of that particular type. Dogs and cats are generally infested by lice, fleas, ticks, and the species used must be fully identified in the report. There are many factors involved in actual use conditions that testing cannot always adequately evaluate. The habits of the host animals, infestation pressures of the parasite, exposure to rain, dew, sunlight, or even pH of water and geographical considerations can result in a change in the degree or duration of efficacy for a particular product. In order to make label claims more meaningful, an accurate description of product performance under a broad spectrum of use conditions should be recorded.

(a) General considerations. (1) Number of trials. A minimum of 5 large-scale, geographically-separated trials are generally necessary, but the number of trials can vary somewhat due to the accessibility of infestations, fluctuations in pest population pressures, behavior, and other important considerations in the biology of the target pest.

(2) Treatment techniques and equipment. Product treatments to control pests of humans are applied as sprays, dusts, lotions, towels, ointments, creams, sticks, soaps, and shampoos. Product treatments to control pests of pets are applied as sprays, dusts, foams, flea collars, flea tags, soaps, and shampoos. For general considerations, refer to § 90-3(a) and (b). For guidance on testing flea and tick collars on dogs, see § 95-30(b) item No. 11.

(3) Evaluation and reporting considerations. Reports should distinguish between killing and repelling effects, since repellent insecticides may preclude any lethal effects. Insect repellency test data should demonstrate minimal contact between the insect and the test chemical. Any adverse effects should be reported. Breed, age, size, and hair length influence the effectiveness of invertebrate control agents applied to dogs and cats, and such factors must be evaluated and reported in testing pet treatments. For general considerations, refer to § 90-3 (d)(1) through (4).

(4) Toxicology tests. Any product intended to be applied directly to humans or pets, or used so as to provide frequent or constant product exposure to humans or pets, must be tested under the full range of toxicology testing required in Subdivision F. After submittal of such toxicology data, consultation with the Agency is required before extensive product performance testing dealing with human pest control products is undertaken.

(b) Suggested performance standards. These standards are presented on the basis of pest population counts from treated compared to untreated subjects unless otherwise specified. (1) Pests of humans. (i) Body louse. Demonstrate 100% control of the stage(s) claimed to be controlled by the test product. Efficacy is based on numbers of the body louse before treatment and at intervals after treatment, in tests conducted where clothing, activities, and environments are typical of those of louse-infested populations. Ovicidal activity must be demonstrated unless repeat applications are intended.

(ii) Head louse and crab louse. Demonstrate 100% control of the stage(s) claimed to be controlled by the test product. Efficacy is based on the numbers of eggs, nymphs and adults of the head louse or crab louse before and after treatment in tests conducted where activities and environments are typical of those of louse-infested populations. Ovicidal activity should be demonstrated unless repeat applications are indicated.

(iii) Ticks, fleas, and mites. Should provide 100% in pest infestation through a killing or repelling action when tested under simulated or actual conditions. Or, the product should provide the protection time (in hours) which is justified by the supporting data and appears on the label. The minimum acceptable protection time is one hour, when using first confirmed bite methodology, or sock testing.

(iv) Mosquitoes. Should generally provide a minimum of 2-3 hours protection time based upon first confirmed bite field tests, depending upon the biting pressure evidenced in the testing. If the product provides longer protection times, then this may be stated on the label. Performance standards for killing mosquitoes are listed under § 95-10 Mosquito, Black Fly, Nonbiting Midge, and Biting Midge (Sand fly) Treatments.

(v) Biting flies. The data should indicate a minimum protection time of 3 hours based upon first confirmed bite field tests. A product may be registered for repelling biting flies if it provides one to three hours of protection, but the direction should be stated on the label.

(2) Pests of pets (dogs, cats, birds). (i) Fleas and ticks. Provide 90% reduction in pest infestation through a killing action when tested under simulated or actual field conditions.

(ii) Lice. Demonstrate 100% reduction in pest infestation through killing or repelling action when tested under simulated or actual field conditions.

(iii) Mites. Provide 100% reduction in pest infestation through killing or repelling action when tested under simulated or actual field conditions.

§ 95-10 Mosquito, black fly, nonbiting midge, and biting midge (sand fly) treatments.

This section is concerned with efficacy data requirements for evaluation of invertebrate control pesticides as outdoor and breeding ground treatments against several groups of important insect pests which pose economic and public health threats to man in urban, rural, agricultural, and wilderness habitats. The insects for which data considerations are outlined here belong to five families: Culicidae (mosquitoes), Simuliidae (black flies), Ceratopogonidae (biting midges), and nonbiting midges in the families Chironomidae (midges) and Chaoboridae (phantom midges). These insects inhabit a variety of ecological niches and show diverse biological, physiological, and behavioral patterns. In the evaluation of biologically active compounds, the unique features of each pest species must be taken into consideration. Members of the 5 families for which these guidelines are developed are mostly aquatic or semi-aquatic in their immature stages. The mature stages are terrestrial, dispersing beyond the immediate vicinity of breeding sources.

(a) General considerations. (1) Species, stage, age, and sex. The test insect should be identified as to genus and species. Additional information as to subspecies or strains should be recorded. Methods and procedures utilized for assessment are dictated by the stage and habitat of the insect. Pesticides are generally evaluated against the larval and/or adult stages. In certain situations, however, registrants may have to gather data on the egg and pupal stages. In larval evaluation, the instar should be standardized and specified. The age or age range (if precise age is not known) of the adult test insects should be reported. Reporting of the calendar age of larvae is not essential if the larval instars are specified. The sex of the adult test insects should be specified. Determination of the sex of the immature stages is difficult, and generally this information is not necessary.

(2) Plot size. Plots which are suitable in size for commercial applications should be used. Plot size can vary from a few square feet to hundreds of acres (hectares), depending on the objectives of the test, species, and type of control desired. Test plot dimensions should be large enough to avoid drift onto sampling areas of untreated control plots.

(3) Number of trials. A minimum of 5 large-scale geographically-separated trials are generally necessary, but the number of trials can vary somewhat due to the accessibility of infestations, fluctuations in pest population pressures, behavior, and other important considerations in the biology of the target pest.

(4) Application techniques and equipment. Aerial or ultra-low volume applications should be evaluated if these methods are specified on the label. For general considerations, refer to § 90-3(a)(3)(ii).

(5) Evaluation and reporting procedures. The evaluation procedures should be specified in the presentation of the data. Reports should include larval counts, bite counts, percent mortality, knock-down time, or other appropriate measures of determining the effectiveness of the test product. Results should include the infestation or nuisance reduction, or preferably both, compared with the control plots or areas. Raw data on pest counts or other measures of control should be summarized. Insect resistance can be a major problem when testing a product for mosquito control, and any evidence of such should be reported when tests are run in areas where resistance is a known problem. Other factors of special concern in determining a product's usefulness are wildlife and fish hazards (see Subdivision E), possible water or air pollution, effects on house paints, and spotting of automobiles. Any adverse effects should be reported. Repellency from mosquito coils, candles, and torches is dependent upon the concentrations and duration of smoke or fumes which permeate a limited area. These types of products should demonstrate repellency of mosquitoes and other small flying insects on patios, torches, or other confined outdoor areas where there is little or no breeze. For general considerations, refer to §§ 90-3(c)(1), (2), and (d)(1) through (4).

(6) Sampling techniques. Various species and the different stages of the same species require specific sampling techniques. Applicable techniques for assessment of populations of test species or groups should be employed.

(b) Suggested performance standards. Unless otherwise specified, these standards are presented on the basis of pre- and post-treatment pest population counts (including landing, bite, and trap counts) from treated as well as the untreated pest plots and standard comparative treatments. All percentages of control refer to the performance of the test product [as determined by pest insect counts (landing and bite) and other measures correlated to insect population pressures] against the vulnerable stage(s) of the target pest, when evaluated according to a recognized aerial or ground application treatment program under actual field conditions.

(1) Culicidae (mosquitoes). (i) Larvae. A minimum of 95% population reduction, based on pre- and post-treatment infestation counts from tests conducted under actual field conditions.

(ii) Adults. A minimum of 95% population reduction, based on pre- and post-treatment infestation counts. When appropriate, laboratory colony or caged wild mosquitoes can be used. The tests should be conducted under actual field conditions.

(2) Simuliidae (black flies). (i) Larvae. A minimum of 80% population reduction, based on laboratory evaluation techniques (jar, cloth trap, flushing and draining, and trough) and the single stream technique to determine effectiveness by comparing larval populations above and below the stream treatment point, not more than one day before and one day after treatment.

(ii) Adults. Large scale control operations are evaluated by pre- and post-treatment population estimates. Landing rate counts (a one-minute count beginning after a 5-minute waiting period is satisfactory), and standardized sweeps about the head of observer inside and outside the treated areas provide a useful index to the annoyance rate.

(3) Chironomidae (midges). (i) Larvae. A minimum of 95% population reduction, based on samples taken prior to treatment, and 4, 7, and 15 days longer after treatment.

(ii) Adults. A minimum of 95% population reduction.

(4) Chaeoboridae (phantom midges). (i) Larvae. A minimum of 95% population reduction, based on larval samples taken prior to and after treatment.

(ii) Adults. A minimum of 95% population reduction.

(5) Ceratopogonidae (biting midges). (i) Larvae. A minimum of 95% population reduction, based on pre- and post-treatment counts. Populations of larvae can be sampled by laboratory inspection of suspected breeding media collected in the field, or by an emergence trapping method to determine the level of control.

(ii) Adults. A minimum of 95% population reduction, based on pre- and post-treatment population estimates determined by landing counts or light trap collections.

§ 95-11 Premises treatments.

This section is concerned with the efficacy data guidance for invertebrate control pesticides used against pests of premises. Commercial and industrial pesticide formulations used for premises treatments include, but are not limited to, liquid or pressurized products for spray treatments and pastes, powders, and granules for baits.

(a) Definitions. The following definitions are of special importance in understanding this section:

(1) The term "premises" refers to the spaces within structures, their walls (both inside and outside), and the immediate adjacent surrounding grounds. Such structures include households (e.g., houses, apartments); commercial, industrial and institutional buildings; agricultural structures (e.g., barns); and food-handling establishments.

(2) The term "food handling establishment" refers to any place, other than a private residence, in which food is held, processed, prepared, and/or served. Food areas include those used for receiving, serving, storing (dry, cold, frozen, raw), packaging (canning, bottling, wrapping, boxing) and preparing (cleaning, slicing, cooking, grinding) food; for edible waste storage; and for enclosed processing systems (mills, dairies, edible oil extractors, and evaporators).

(3) The term "non-food areas" refers to premise areas such as garbage rooms, lavatories, floor drains (to sewers), entries and vestibules, offices, locker rooms, machine rooms, boiler rooms, garages, mop closets, and storage (after packaging.)

(4) The term "crack and crevice treatment" means the application of small amounts of insecticide into cracks and crevices in which insects hide or through which they may enter the building. Such openings commonly occur at expansion joints, between different elements of construction, and between equipment and floors. These openings may lead to voids such as hollow walls, equipment legs and bases, conduits, motor housing, junction or switch boxes

(5) The term "spot treatment" is application to limited areas on which insects are likely to occur, but which will not be in contact with food or untensils and will not ordinarily be contacted by workers. These areas may occur on floors, walls, and bases or undersides of equipment. For this purpose, a "spot" will not exceed 2 square feet. Spot treatments must be limited to 20% of the lower wall and floor surfaces. The 2 square foot "spots" cannot be contiguous.

(6) The term "general treatment" is application to broad expanses of surfaces such as walls, floors, and ceilings or as an outside treatment.

(b) General considerations. The testing of a pesticide product for use in households should follow the same procedures as are appropriate for its commercial use.

(1) Site selection. The uniqueness of each application site or structure, and the fact that identical sites or structures will usually not be available, should be recognized. For general considerations, refer to § 90-3(a) and (e)(3)(vi).

(2) Number of trials. Under controlled conditions in the laboratory, at least 5 trials are usually necessary. A minmum of 5 large-scale field trials are generally needed, but the number of trials can vary somewhat due to the accessibility of infestations, fluctuations in pest population pressures, behavior, and other important considerations in the biology of the target pest.

(3) Test species. Test invertebrates should be representative of flying and/or crawling species against which the test product is to be used. A pest population may be difficult to estimate or to sample, and it will often be necessary to limit testing to the laboratory, or to develop a system of estimating the extent of an infestation in the field. The pests to be controlled often cannot be distinguished as separate entities and will frequently need to be grouped according to control methods, geographical locations, infested premises, life stage or form, type of damage and other factors. Therefore, testing may need to be directed at the most important pest or pests, life stage or pest complex, or at the species most resistant to a particular pesticide.

(4) Exposure period. The duration of exposure to the test product is important in order to achieve maximum control, and data must be reported in terms of time length, such as 12-, 24-, or 48-hour exposure periods.

(5) Temperature and humidity. The temperature and humidity at the time the product is tested should be recorded, since these factors are important relative to the activity of the pest population, and the degradation of the active ingredient(s).

(6) Residual considerations. The amount of pesticide residue deposited on treated surfaces is critical to the effectiveness of many treatments against crawling pests. The amount of residue deposited should be determined under actual or simulated use conditions, and the method(s) of determination must be submitted with the test data. The types of surfaces to which residual pesticides are applied must be reported since surface type has a pronounced effect on the amount of active residue available to pests. In general, the following absorptive and non-absorptive surfaces should be tested for crawling pests: vinyl tile or linoleum, stainless steel, painted and unpainted wood and ceramic tile.

(7) Application techniques and equipment. The application technique should reflect the claims proposed on the label, whether crack and crevice, spot, general, space spray, contact spray or total release. Insecticides to be evaluated in or around dairy barns, horse barns, stables, poultry houses and yards, and other agricultrual structures may be applied as sprays, dusts, and baits to stanchions, walls, floors, manure, bedding, and other areas where the target insects may rest or breed. For general considerations, refer to § 90-3(a).

(c) Test methods and suggested performance standards. These suggested standards are determined on the basis of results obtained from laboratory and simulated or actual field tests in which the efficacy of the test product is compared with that of official test pesticides such as the official test pressurized spray (OTPS), from the Chemical Specialties Manufacturers Association, untreated controls, or a registered standard. The performance standards are the same for household, commercial and industrial, agricultural, structural and food handling establishment treatments.

(1) Non-residual aerosols and space sprays. (i) Flying insects. The product should demonstrate efficacy equal to, greater than, or within 5 percentage points of the OTPS when tested according to the CSMA Aerosol and Pressurized Spray Insecticide Test Method for flying insects or the ANSI-ASTM E653-78 Standard Method for Testing Effectiveness of Aerosol and Pressurized Space Spray Insecticides Against Flying Insects.

(ii) Crawling insect. (A) Contact spray. The product should be tested against the proposed target pest and demonstrate performance equal to, greater than, or within 10 percentage points of the OTPS when tested according to the CSMA Cockroach Aerosol Method or the ANSI-ASTM E654-78 Standard Method for Testing the Effectiveness of Aerosol and Pressurized Spray Insecticides Against Cockroaches.

(B) Space spray. There are no official test methods for space sprays. However, the following general considerations apply to pesticides applied as space sprays for crawling insects, as most of these insects are not exposed at the time of application.

(a) The room size and dosage must be consistent with label claims and directions.

(b) Replication is necessary to provide reliability.

(c) Controls should be utilized for comparison.

(d) As some products may knockdown but not kill a high percentage of insects, mortality must be recorded at 1 and 24 hrs. post treatment.

(e) Usually German cockroaches are used in testing. When dosage rates are very small, a larger species, such as American or Oriental cockroaches, may also be necessary. Resistant strains should be utilized if the pesticide has a history of resistance problems. Last instar nymphs and adults are recommended for all species.

(f) A product registered for the use pattern should be used as a positive control.

(g) The containers or enclosures for holding the roaches should be representative of these areas which commonly shelter roaches (underneath refrigerators, stoves, sinks, cabinets and in certain wall voids.

(h) The number of specimens should be sufficient to provide accurate statistical evaluation of the results. Perhaps the greatest problems have arisen with respect to item (g) above. Note that open containers or those with unobstructed holes in the top area are not representative of covered or enclosed situations.

(2) Residual sprays. (i) General: Efficacy data for residual sprays should indicate the appropriate dosage and the utility of the formulation when used as directed. Usually, laboratory testing is performed to establish the effective dosage range, determine if the formulation is repellent to the point of adversely effecting performance of the product, and to evaluate the effects of the various substrates upon which deposition is to occur. Field studies are then necessary to confirm the performance of the compound under actual use conditions. See § 95-30 items 5 and 12.

(ii) Sprays. (A) Crawling insects. (a) Laboratory testing. Laboratory testing should evaluate the proper dosage range utilizing treated panels representative of field substrates. Usually these include such items as painted and unpainted wood, glass, formica, chipboard or particle board, stainless steel, concrete, and vinyl tile. Such treated panels should be aged under conditions similar to field situations and challenged with the target insect as regular intervals to determine the duration of efficacy. Additionally, choice box testing or some other method of evaluation must be performed to determine if a high degree of repellence is likely to affect field performance. All laboratory testing should utilize both untreated and positive controls to determine the relative vigor of the laboratory populations.

(b) Field testing. Field testing for crawling insects involves utilizing sites and methods of application essentially the same as those to appear on the proposed label. Evaluation is based upon pre- and post-application counts of living insects. For example, cockroaches are normally counted by flashlight, trapping, or flushing agents. Untreated areas or sites should be used as controls if possible, and a positive control should also be used in the testing.

(B) Flying insects. (a) Lab tests. Laboratory tests for residual applications to control flying insects are usually done in screen cages with one side replaced by the treated panel. Such testing should evaluate the appropriate dosage range (usually mg. active ingredient per square foot), and the degree of repellency exhibited by the formulation.

(b) Field testing. Field tests should utilize the proposed sites of application. Counts may be made by visual observation, fly speck cards, black light traps, or other suitable methods.

(3) Baits. (a) General. The important factors relating to testing bait products are to:

(1) Establish the proper dosage and intrinsic attractancy of the formulation in "free-choice" laboratory tests.

(2) Evaluate the utility of the product under actual-use conditions. See § 95-30 items No. 5 and 12.

(b) Laboratory testing. The most important factor involved in lab testing is to provide a free-choice alternative food source to the test insects. This may be laboratory dog chow for insects like cockroaches, or sugar based materials for houseflies. The formulation should demonstrate acceptable toxicity in competition with the alternative food source.

(c) Field testing. Testing under actual use conditions should demonstrate the utility of the product when used in situations where a continuous source of pest insects are available. The product should demonstrate both mortality and a reduction in the overall population over time. Measurement techniques are essentially the same as for residual sprays.

§ 95-12 Structural treatments.

This section concerns the guidance on efficacy data for evaluation of invertebrate control pesticides used to control pests such as wood-destroying beetles, wharf borer, carpenter ant, and carpenter bee in structures such as buildings, structural members, and stored lumber.

(a) General considerations. (1) Site selection. When selecting an application site for testing invertebrate control agents against structural pests, such factors as temperature, humidity, moisture, soil texture, and freezing-thawing conditions should be considered and reported. For general considerations, refer to §§ 90-3 (a)(3)(iv) and (b)(1) through (3).

(2) Number of trials. A minimum of 3 large-scale geographically-separated trials are generally necessary, but the number of trials can vary somewhat due to the accessibility of infestations, fluctuations in pest population pressures, behavior, and other important considerations in the biology of the target pest.

(3) Residual considerations. The soil residual potential for termite control products is important in determining the length of time that such pesticides will be effective, and is measured by the time over which the toxic barrier resists penetration. Soil treatments require that special care be exercised to assure that water supplies are not contaminated with the pesticide and that the treatment does not cause intolerable injury to building foundations, plants, shrubs, and other ornamental plants.

(4) Application techniques and equipment. Data should be developed to support the use of pressurized, impregnation, or other specialized types of application equipment: For general considerations, refer to § 90-3 (a)(3)(i).

(5) Evaluation and reporting procedures. (i) The procedures used to evaluate product performance should be specified in the data. Reports should include insect counts, amount of damage, or to the measures of determining the effectiveness of the test product. Results should include the average infestation reduction throughout the sampled post-treatment period compared with that in untreated controls. Data on pest counts or other measures of control should be summarized. The test data should indicate that there are no adverse effects such as warping or staining to wallpaper, paint, tile, varnish, or other surfaces if they are likely to

be contacted by the product when applied according to label directions for use. The data should also indicate that the material does not impart long-lasting objectionable odors to furniture, cabinets, or other indoor treatment sites which may be specified on the label.

(ii) Wood-destroying beetles include species of Anobiidae, Lyctidae, Bostrichidae, and one species of Cerambycidea: Hylotrupes bajulus (the old house borer), all which may reinfest structural members of lumber in storage. Tests for use of wood preservatives to control adults of powder-post beetles, old house borer, carpenter ants, and certain other wood-destroying insects should follow conventional test method procedures by treatments designed for the control of flying insects; and these could be evaluated by tests paralleling those for other household pests.

(iii) Wharf borer adults emerging into buildings or in areas around the sites of former buildings are controlled by treatments designed for the control of flying insects, and these are evaluated by tests paralleling those for other household pests. [Refer to § 95-11].

(iv) Subterranean termite attacks in a structure are prevented or controlled through impregnation of soil with a termite toxicant beneath and adjacent to the structure. Protection of wood from attack by subterranean termites may also be provided by impregnation of the wood by a termite toxicant.

(v) Dampwood termites are evaluated by the same procedures that are practical for the control of subterranean termites. [Refer to paragraphs (b)(1)(i)(B) and (b)(1)(ii)(B) of this section].

(vi) Drywood termites are controlled by fumigation or treatment of channels with dusts, liquids, or spot fumigants for the elimination of drywood termites should be evaluated by observing the effect of such treatments under field conditions. Absorptive dusts have been used as a protective barrier treatment and should be evaluated on this basis in field treatments. For general considerations, refer to §§ 90-3 (c)(2) and (d)(1) through (4).

(b) Specific considerations and suggested performance standards. (1) Subterranean termites are by far the most important structural pests. These insects damage wood and other cellulosic building materials causing major property damage particularly in the southern areas of the United States. Control procedures usually are reflective of the fact that colonies of these termites require contact with the soil as opposed to drywood or dampwood termites which may exist independently of soil moisture.

(i) Preventive treatments - soil barriers. (A) Data considerations. Subterranean termite attacks in a structure are prevented or controlled through toxicant impregnation of the soil beneath and adjacent to the structure. Data should indicate that the treatment does not cause intolerable injury to building foundations, plants, shrubs, or to other ornamentals. For general considerations, refer to § 90-3 (c)(1). The effectiveness of prophylactic termite treatments is measured by the time over which the toxic barrier remains effective in resisting penetration by the termites. Therefore, the efficacy data should indicate the establishment and maintenance of this toxic barrier.

(B) Test methods. Published laboratory methods are not currently recognized by the Agency as giving a reliable evaluation of soil toxicants for termite control, but three field tests are recognized as giving a reliable evaluation of soil toxicants: the Stake method, the Ground board method, and the Modified ground board method.

(C) Suggested performance standards. Data derived from such testing should provide complete resistance to termite attack for a period of 5 years, based upon annual reinspection. The tests should be in geographic areas which provide year-around pest pressure (usually in the southern U.S.).

(ii) Preventive treatments - wood impregnation. (A) Data considerations. Subterranean termite damage in a structure is also prevented by treatment of susceptible materials. These treatments are applied as dips, brush-on applications, sprays, or pressure treatments. The activity of the materials is such that damage to the treated cellulosic substance is precluded or reduced either because the treated material is toxic upon ingestion or sufficiently repellent that ingestion does not occur. The effectiveness of such treatments is determined by a measurement of the duration for which the treated material resists termite attack.

(B) Test methods. Modifications of the Stake method, Ground board method, and Modified ground board method are acceptable in evaluating impregnated wood products.

(C) Suggested performance standards. When acceptable data derived from testing for at least 2 years but less than 5 years shows a complete resistance to termite attack, the product may be registered contingent upon a restriction which specifies annual reinspection. The tests should be conducted in geographic areas which provide year round pest pressure (usually in southern U.S.).

(iii) Infested sites. (A) Data considerations. Data should be derived from experiments utilizing field infestations. These experiments must address the considerations contained in § 90-3 and evaluate the effects of the treatment as related to the infested site.

(B) Test methods. (Reserved).

(C) Suggested performance standards. Data should indicate not only that the insects are killed, but that those insects in egg and nymphal instars at the time of treatment will be controlled either by immediate or residual toxicity. The data should clearly indicate that the entire colony is destroyed by isolation from the essential water source by the establishment of a pesticidal barrier, through the penetration of all wood galleries by the pesticide, or by both routes.

(2) Dampwood termites and drywood termites. Dampwood termites may exist in structural members where a water source is constantly available to the colony due to faulty plumbing, roof leaks, or other factors. Drywood termites can colonize in wood or other cellulosic substances regardless of any apparent water source, since they are capable of metabolizing sufficient moisture from the materials they ingest. For this reason, applications

of pesticides for the control of these pests are usually preventative or eradicant treatments of actual infested material. These wood treatments generally parallel those for subterranean termites, and paragraphs (b)(1)(ii) and (b)(1)(iii) of this section address the data considerations, testing procedures, and performance standards which are also applicable to compounds used against dampwood and drywood termites.

(3) Other terrestrial pests of structures. These include insects such as powder post beetles, the old house borer, carpenter ants, carpenter bees, longhorned borers, the furniture beetle, and the wharf borer. The biological characteristics, sites of infestation, types of damage and treatments used for control of these pests are complex and often complicated in nature. Data to support use patterns for these pests will be considered on a case-by-case basis when applicable, until specific data considerations are made available.

(4) Structural pests in aquatic sites. (Reserved).

§ 95-13 Stored product treatments.

This section concerns the data considerations for the determination of the effectiveness of invertebrate control pesticides against pests of stored products. These include the confused flour beetle, rice weevil, lesser grain borer, Indian meal moth, cowpea weevil, Angoumois grain moth, cadelle, granary weevil, sawtoothed grain beetle, and other stored product insects. These pests should be controlled not only in the stored product commodities but also in facilities where the commodities are stored, processed, manufactured, packaged, and transported. Some species cause damage in both the larval and adult stages. See § 95-30 reference no. 13 for examples of methods for evaluating stored-grain insect pests.

(a) General considerations. The testing of a pesticide product for household use should follow the same procedures as are appropriate for the product's commercial use.

(1) Site selection. Commodities to be protected include grain, seeds, cereals and other dry foods, tobacco, woolen and feather goods, furs, and furniture stored in such sites as grain elevators, boxcars, ship's holds, and warehouses. The types of commodities and packaging around which the test product is to be tested must be representative of the types of commodities and packaging in areas in which the product is intended to be used, such as pantries, storage closets, warehouses, and other areas. Test sites should be selected which are representative of pest populations commonly encountered. Other factors such as climate and geographic location should also be considered and reported. For general considerations, refer to § 90-3 (a)(3)(iv) and (b)(1), and (3).

(2) Number of trials. A minimum of 5 large-scale, geographically-separated trials are generally necessary, but the number of trials can vary somewhat due to the accessibility of infestations, fluctuations in pest population pressures, behavior, and other important considerations in the biology of the target pest.

(3) Exposure time. The length of the treatment exposure time should be stated in terms of time length such as 12, 24, or 48 hours, depending on the formulation of the test product and the pest species involved.

(4) Temperature and humidity. Temperature and humidity should be reported in the data since they can affect the activity of the pest species and the effectiveness of the test product. This is particularly true when testing fumigants.

(5) Residual considerations. The residual activity of the pesticide product should be evaluated to validate control over periods of days and weeks. Pesticides to be registered for use on stored food products are required to have a residue tolerance level set or they must have an exemption from the requirement for a tolerance, as stipulated in the Federal Food, Drug and Cosmetic Act (21 U.S.C. 346, 346a, and 348).

(6) Application techniques and equipment. Ultra-low volume application equipment or fumigation techniques should be evaluated if these methods are specified on the label.

(7) Evaluation and reporting procedures. The procedures for evaluating product performance should be specified in the presentation of the data. Reports should include insect counts, amounts of damage, or other measures of determining the effectiveness of the test product. Results should include the average pest infestation reduction compared with with that in the controls, and an assessment of any possible adverse effects on the grade or quality of the treated commodity. Raw data on pest counts or other measures of control should be summarized and submitted. Tests should be conducted on palatability in all cases where food or feed germination or viability should be provided. Space sprays and residual treatments should be evaluated under conditions which minimize the contamination of stored feed, food, or tobacco. Tolerances for stored food and feed should not be exceeded. For general considerations, refer to § 90-3 (c)(2) and (d)(1) through (4).

(b) Suggested performance standards. These suggested standards are determined on the basis of results obtained from laboratory and simulated or actual field tests in which the efficacy of the test product is evaluated against products of known efficacy used as reference standards, or against untreated controls. The following stored products pests are covered: confused flour beetle, rice weevil, lesser grain borer, Indian meal moth, cowpea weevil, Angoumois grain moth, cadelle, granary weevil, sawtoothed grain beetle and other stored product insects.

(1) Aerosols. Aerosol products should demonstrate efficacy equal to or better than the official test aerosol (OTA).

(2) Fumigants. Fumigant products should demonstrate efficacy comparable to a registered standard such as methyl bromide.

(3) Insect resistant packaging. Insect resistant packaging should demonstrate complete protection against stored product pests. Control is based

on representative packages which should be examined for insect penetration, and for insects in the commodity, at frequent intervals throughout the tests.

(4) Residual protectants. Residual protectants should demonstrate efficacy comparable to a registered standard such as malathion.

(5) Vapors. (Reserved).

§ 95-14 Fabric treatments.

This section concerns the data considerations for efficacy evaluation of pesticide products used for control of invertebrate pests of fabrics. The principle pests of fabrics include the webbing clothes moth and black carpet beetle.

(a) Definitions. The following definitions and explanations are of special importance in understanding this section:

(1) The term "fabrics" refer to all animal, plant, and synthetic fibers or blends to such materials.

(2) The term "temporary fabric treatments" refers to non-residual spray applications which kill fabric pests by contact action. Such treatments may require repeated application.

(3) The term "semipermanent fabric treatments" refers to residual chemical applications to wool, fur, or hair rugs, garments and blankets as coarse sprays or, in some cases, in dry cleaning solutions. To prepare for semipermanent mothproofing tests, the articles must be sprayed lightly but uniformly until the surfaces are moist but never soaked or saturated, and the sprayed articles thoroughly dried before testing.

(4) The term "permanent fabric treatments" refers to residual chemical impregnation in the fabric during the dye process. Test to determine effectiveness of these treatments require that the impregnated fabrics be tested before and after repreated washing, drycleaning, and other tests such as the performance tests of the American Association of Textile Chemists and Colorists.

(b) General considerations. The testing of a pesticide for household use should follow the same procedures as are appropriate for its commercial use.

(1) Test species. Although several species of insects attack woolen fabric and other animal fibers, the webbing clothes moth and black carpet beetle should be used in conducting efficacy tests for fabric protectants.

(2) Number of test insects. The number of test insects used is important in obtaining an accurate evaluation of these types of treatments, and at least 120 insects of each pest stage (larvae and adults) should be used for each test when evaluating temporary fabric protectants (direct contact sprays). When evaluating semipermanent and permanent fabric protectants,

use the number of insects set forth in the official CSMA "Textile Resistance Test", and the American Association of Textile Chemists and Colorists test method 24-1977.

(3) Number of trials. Four large-scale trials are generally preferred, and the number of trials can vary somewhat due to the accessibility of infestations, fluctuations in the pest population pressures, behavior, and other important considerations in the biology of the target pest. Because of the nature of fabric-insect problems, large scale trials are often impossible to conduct. However, for permanent fabric treatments, laboratory application data should be supplemented by pilot plant or mill scale application data.

(4) Observations. For agents providing temporary mothproofing, the test insects should be observed 24 and 48 hours after treatment and the percentage of knocked-down (KD) and dead plus moribund (D+M) insects recorded. Data should be presented in tabular form. When testing semi-permanent mothproofing products, the appropriate excrement-weight or fabric-weight loss data, mortality data, and the analytical results should be submitted in tabular form. Visual feeding damage ratings are useful.

(5) Application techniques and equipment. The data obtained must reflect the effectiveness of the test product when applied in commercial dry-cleaning and dying equipment. For general considerations, refer to § 90-5 (a)(3)(i).

(6) Evaluation and reporting procedures. The procedures used to evaluate product performance should be specified in the data. Reports should include mortality, fabric weight loss, excrement weight, analytical results, or other measures of determining the effectiveness of the test product. Data obtained should be based on the effect of the test product in protecting fabrics from damage by the larval stage of the pests. All observed adverse effects of the test product should be reported. For general considerations, refer to §§ 90-3 (c)(2) and (d)(1) through (4).

(c) Suggested performance standards. These suggested standards are determined on the basis of results obtained from laboratory tests following procedures set forth in the official CSMA "Textile Resistance Test," and the American Association of Textile Chemist and Colorists standard test methods 24-1977 and 28-1977. The performance standards cover the following fabric pests: webbing clothes moth and black carpet beetle.

(1) Temporary fabric treatments. Non-residual products that should provide almost complete (99%) control under repeated treatments every 30 days.

(2) Semi-permanent fabric treatments. Residual products should provide protection of the fabric as specified by CSMA and the American Association of Textile Chemists and Colorists test methods for 6 months to one year.

(3) Permanent fabric treatments. Residual products which are impregnated into the fabric during the dyeing process should provide protection of the fabric as specified by CSMA and the American Association of Textile Chemists and Colorists before and after the performance tests listed below:

Manipulation	Minimum requirements	Maximum requirements
Washing	5 times	10-15 times
Drycleaning - Stoddard solvent or perchloroethylene	5 times	10-15 times
Hot pressing	5 times	10-15 times
Sea water	5 immersions	
Perspiration:		
acid -	5 immersions	
alkaline -	5 immersions	
Light: Fade-Ometer	40 Standard Fading hours	100 Standard Fading hours
Abrasion	2000 revolutions - wear test machine 2000 revolutions for 6 minutes - Accelerotor	

§ 95-30 Acceptable methods

The following thirteen items contain acceptable protocols for the testing of invertebrate control agents.

(a) The following 10 items are booklets prepared for the Agency by eminent scientists in each discipline, under contract through the American Institute of Biological Sciences (AIBS), and all booklets are available from the National Technical Information Service (NTIS), 4285 Port Royal Road, Springfield, VA 22151.

(1) Anonymous. 1977. Analysis of specialized pesticide problems. Invertebrate control agents. Efficacy test methods. Vol. I. Foliar treatments I. Deciduous fruit trees, small fruits, citrus and subtropical fruits, tree nuts. EPA contract number 68-01-2457, AIBS, Arlington, VA. 80 pp.

(2) Anonymous. 1977. Analysis of specialized pesticide problems. Invertebrate control agents. Efficacy test methods. Vol. II. Foliar treatments II. Field crops, forage crops, rangeland, vegetables--field and greenhouse. EPA contract number 68-01-2457, AIBS, Arlington, VA. 137 pp.

(3) Anonymous. 1977. Analysis of specialized pesticide problems. Invertebrate control agents. Efficacy test methods. Vol. III. General soil treatments. EPA contract number 68-01-2457, AIBS, Arlington, VA. 99 pp.

(4) Anonymous. 1977. Analysis of specialized pesticide problems. Invertebrate control agents. Efficacy test methods. Vol. IV. Livestock, poultry, fur, and wool-bearing animals. EPA contract number 68-01-2457, AIBS, Arlington, VA. 68 pp.

(5) Anonymous. 1977. Analysis of specialized problems. Invertebrate control agents. Efficacy test methods. Vol. V. Stored products and premise treatments. EPA contract number 68-01-2457, AIBS, Arlington, VA. 58 pp.

(6) Anonymous. 1977. Analysis of specialized problems. Invertebrate control agents. Efficacy test methods. Vol. VI. Lawns, ornamentals, forest lands. EPA contract number 68-01-2457, AIBS, Arlington, VA. 55 pp.

(7) Anonymous. 1977. Analysis of specialized problems. Invertebrate control agents. Efficacy test methods. Vol. VII. Human and pet treatments. EPA contract number 68-01-2457, AIBS, Arlington, VA. 24 pp.

(8) Anonymous. 1977. Analysis of specialized pesticide problems. Invertebrate control agents. Efficacy test methods. Vol. VIII. Mosquitoes, black flies, midges and sand flies. EPA contract number 68-01-2457, AIBS, Arlington, VA. 52 pp.

(9) Anonymous. 1978. Analysis of specialized problems. Invertebrate control agents. IX. Baculoviruses and entomogenous bacteria. EPA contract number 68-01-2457, AIBS, Arlington, VA. 115 pp.

(10) Anonymous. 1978. Analysis of specialized pesticide problems. Invertebrate control agents. Efficacy test methods. Vol. X. Turf, ornamentals, forest lands. EPA contract number 68-012457, AIBS, Arlington, VA. 81 pp.

(b) Items (11) and (12) are adapted from guidance documents prepared by scientists in the Agency's Registration Division of the Office of Pesticide Programs.

(11) Guidance for the testing of flea and tick collars for control of ticks on dogs.

1. Scope. There are many commercially produced pesticidal matrices (as collars and tags) which may now or in the future bear label claims for the control of fleas or ticks. Such claims are acceptable only upon a demonstration of the efficacy of the product, as claimed, when used according to label directions. The strength and kind of acceptable label claims are generally determined by four factors which will be discussed later in greater detail. These factors are:

1.1 The degree of control against fleas and ticks.

1.2 The anatomical coverage of the product.

1.3 The species of parasites controlled.

1.4 The duration of the control afforded.

This protocol is designed to aid the investigator or registrant in the development of data as it applies to the registration of various claims for impregnated products, in such a manner as to include all of the above mentioned considerations.

2. Definitions:

2.1 Control animals - Those animals not treated with any pesticide product.

2.2 Test collar animals - Those animals wearing the collar which is under evaluation.

2.3 Placebo - A collar formulated from the same carrier matrix as the collar to be tested, but lacking any active pesticide ingredients.

2.4 Qualified personnel - Individuals who count or apply the reinfestations and are trained in finding and recognizing the parasites on the dog. They should also be familiar with the biology of the parasite and the recording techniques necessary in development of meaningful data.

2.5 Positive control - A collar registered for the proposed label claims.

3. Definition of control. The magnitude of the infestation on the control animals should be representative of the population as established by the screening procedures. Test collar dogs will then be compared to the controls by parasite count and body areas infested. The acceptance of a collar for registration and ultimate control claims allowed will be dependent on a number of factors. These may include the overall number of fleas or ticks, the number of live attached ticks and live fleas on the test collar dogs in comparison to the control animals; and the positive control animals.

4. Size of the test. The test should be large enough to permit good statistical evaluation. The exact number of animals bo be evaluated cannot be specifically stated. However, the investigator is reminded that the validity of the resulsts of the testing program is directly related to the degree of variability within the test. Increasing the number of test animals increases the reliability of the test results. In general, a minimum of six animals per group is required, with 10 preferred in any single test.

5. Selection of test arthropods.

a. Ticks. Limited or single species testing may be utilized to support restricted claims. For general tick control claims, however, testing should be designed to show sufficient biological activity to control those species of ticks common to dogs. Therefore, a minimum amount of testing should be conducted to ascertain sufficient activity against representative tick species. In order to support a general tick claim, data must be derived on the brown dog tick (*Rhipicephalus sanguineus*) and at least one other species of tick common to dogs. In addition to the brown dog tick, other tick species common to dogs include the lone star tick (*Amblyoma americana*), American dog tick (*Dermacentor variabilis*) and the Rocky Mountain wood tick (*Dermacentor andersoni*). *D. variabilis* is preferred.

b. Fleas. The only species required to be tested is the cat flea, *Ctenocephalides felis*. This flea is the predominant species on both dogs and cats. Mixed flea populations may be used providing that an adequate infestation of *C. felis* is established on the test animals and the fleas of different species are counted and recorded separately.

6. Testing specifications.

6.1 All testing shall be conducted on male and female adult dogs of various sizes and hair lengths.

6.2 Placebo collars shall be used on the control animals.

6.3 The animals should have a clean bill of health substantiated by a veterinarian and should undergo a preconditioning period of at least seven days before use. All animals should be washed with a non-residual insecticidal shampoo before initiation of the testing.

6.4 All control and test collar dogs shall be prescreened for tick attachment before initiation of the test. Only animals showing acceptable tick attachment shall be used in the study.

6.5 Any other insecticides used on or near the animals during the course of any testing period must be fully justified, including any effects of such application on tick populations.

6.6 While not required, a positive control test using a collar registered for tick control would serve as a standard reference and aid in the final evaluation of the test collars.

6.7 A minimum of 5 large dogs (40 lbs.) must be represented in the sum of the entire series of tests submitted for registration. It is recommended, that at least 3 large dogs be included in each group in each test.

7. Exposure.

7.1 Adult parasites are recommended for infestation of the control and test collar animals. All ticks should be of uniform age and feeding status (unfed) since the last moult.

7.2 There are several basic methods for infesting dogs with fleas and ticks. Any of these may be acceptable in developing data for registration. Regardless of the infestation procedure, the primary consideration is to achive adequate numbers of attached parasites with sufficient anatomical distribution to allow evaluation of collar effectiveness. The infestation procedure should be refined to the extent that equal infestation pressures are applied to both control and test collar dogs. In general, a minimum of 3 ticks and/or 5 fleas per dog (controls) are necessary for a valid test.

7.3 All animals should be housed individually in holding enclosures. The holding environment should provide both indoor and outdoor exposure.

7.4 If the proposed label claims refer to the ability of the collar such that performance is unaffected by washing, then the test collared animals should be bathed biweekly with a non-insecticidal shampoo.

8. Reinfestation.

8.1 Since fleas and ticks will leave a dog in certain situations regardless of the presence of a collar, a reinfestation of unfed parasites must be made at specific intervals during the testing. All animals should be reinfested periodically following the initial infestation for the entire period of control proposed for the product. Frequent observations as described below for the initial infestation should be recorded for each subsequent reinfestation. The same period of observation (1-7 days) should be utilized for these reinfestations, and all remaining parasites should be removed at the end of each counting period. The duration of testing must be consistent with label claims; therefore, infestation pressure must be maintained throughout the entire period of control. It is suggested that the actual testing be continued in excess of the label claim being sought to provide more confidence in the data derived.

8.2 The reinfestation pressure should be of sufficient degree to induce an acceptable quantity of parasites to attach to the control animals.

8.3 The same collar shall be used on an animal for the duration of a study, and control and test collar animals shall not be interchanged prior to a reinfestation challenge.

9. Observations.

9.1 All observations should be made and records maintained by qualified personnel.

9.2 A pretreatment inspection of all animals should be made to ascertain the number of parasites on the dogs prior to the installation of the collars.

9.3 The initial observations should be made soon after inoculation to ascertain the knockdown potential of the collar. This observation (usually at 2 hours, once again at 8 hours) is essential for a "fast acting" claim if so desired by the registrant. It is realized that observations made this soon after inoculation may affect the degree of attachment for the first counting period.

9.4 Frequent counts of parasites are then recorded (e.g., every 2-3 days) after infestation for a period of 5-7 days or until substantial detachment occurs after engorgement. These inspections should indicate the number, sex, attachment and viability (living or dead) of the ticks along with the specific locations on the animals (head, neck, torso, rump, legs, feet). This information should be recorded in conjunction with the age of the collar for the particular day on which a dog was inspected. For fleas; only the number of parasites need be recorded.

9.5 Records should be kept for each individual animal, and the data combined into tables which compare the various test groups (test collars, controls, positive control animals).

9.6 All parasites remaining on animal (both test collar and control) at the end of the counting period should be removed as well as possible.

9.7 All abnormal signs and symptoms during the course of the study shall be noted and recorded for both control and test collar animals.

10. Evaluation and interpretation of data derived from testing. The strength and kind of acceptable level claims are generally determined by four factors:

10.1 The degree of control.

10.2 Anatomical sites of tick attachment.

10.3 The species of parasites tested.

10.4 The duration of effectiveness.

Ticks. First is the degree of control afforded by the collar. Such claims as "control ticks" must be supported by data which indicate that the collar will effectively reduce substantial populations of ticks to a level significantly below that of the control. For all practical purposes, this would be control to the extent that the user would not usually be required to rely on other pesticides or methods of control during the time period specified on the labeling. It is realized that 100% tick control will rarely be achieved in tests where the outdoor exposure may provide for a source of additional reinfestation. In general, 90% control is a desirable level of reduction, but even 80% control may support label claims under certain circumstances.

It is also realized that some benefit is derived from collars which do not give control of ticks, yet induce a consistent recognizable reduction in the number of ticks on the test animals as compared to the control animals. Label claims for "aids in the control of ticks" are appropriate for such products. A recognizable reduction is a level of protection which is evident not only upon statistical analysis but which would be apparent to the purchaser of the product in actual use situations. A minimum of 50% reduction must be apparent for an "aids in control" claim.

The second area of consideration is the use of anatomical restriction in those cases where the concentrations of pesticide generated from the collar matrix do not control ticks over the entire body of the dog. If anatomical restrictions are necessary, they will be added to the tick claim. A familiar example could be to specify control "in the head and neck area only."

As stated in section 5.a., the species of ticks tested will determine the label claims to be supported.

The final factor involved in the interpretation of the data is the duration of testing as compared with efficacy. Label claims must be justified by data which are derived from testing the collar for that period of time which is to appear on product labeling. Such data must at least show the collar to be efficacious over the control period proposed for the product.

Fleas. For fleas, a minimum of 90% control, as compared to the counts on the placebo animals, is required for the duration of testing. There are no "aids in control" claims or anatomical restrictions in regard to claims for the control of fleas.

11. Other data.

Note that other kinds of data are also considered when evaluating the efficacy of a product. Such information as pesticide release rates, when accurately measured and correlated with the effective dosage of the active ingredient to ticks, may also be used. Such data should be derived from in vivo tests and reported as mg. active ingredient released per day. Additionally, consumer or veterinary testing is often utilized to further indicate the performance of a collar.

This provisional protocol is not designed to be the only acceptable test methodolgy for the generation of applicable data. It is merely one acceptable method which attempts to resolve many of those items which have resulted in data which were unacceptable to support registration in the past.

(12) Cockroach bait test method.

I. Introduction.

This is a suggested test procedure for cockroach baits to be used to control cockroaches in homes, warehouses, food establishments, etc. The following outline is a suggested series of laboratory and field tests. The important factors are to:

1. Establish the proper dosage and intrinsic attractancy of the formulation in "free-choice" laboratory tests.

2. Evaluate the utility of the product under actual-use conditions.

II. Test protocol.

A. Choice box laboratory test.

1. Enclosures (4) constructed out of plywood. Dimensions approximately 2'x4'x4". Tops are either made of plexiglass or the sides are greased or teflon coated to prevent escape. Each enclosure is divided into two halves by a line drawn or painted down the center giving two 2'x2' areas. In each side are placed a cardboard hide (12"x12"x12" ht. is suggested) and water. In three of the enclosures, one side is treated with the proposed dosage of bait (gm. per unit area) and on the other side an equal amount of Purina Dog Chow (R) is placed. Some of the bait and dog chow may be placed in the hides. In the remaining box only Purina Dog Chow is used as a control. Each box receives 50 last instar nymph or adult German cockroaches, Blatella germanica placed either along the center line or 25 in each side. Boxes are kept in areas providing a normal day/night photoperiod or a minimum of 8 hours darkness each day. Roaches are counted at 1, 3, 5, 7, 10, 14 and 21 days and the percent mortality can be derived from Abbott's formula. A table of results and the counts for the individual boxes should be submitted. Consumption may indicate that additional bait is necessary to give acceptable mortality. If all the bait is consumed, then additional bait or dog chow may be added as necessary. Such additions must be recorded and reported. Testing may need to be repeated in situations where definitive results are not obtained.

2. Ovicidal and chemosterilant action-trials may be designed to indicate either a chemosterilant effect or toxicity to the eggs carried by the female. For chemosterilants, the laboratory choice box test should be continued for as many days as necessary to collect significant numbers of oothecae. Upon deposition, these should be collected from the boxes, and kept in separate

containers. Percent eclosion should be recorded for treated boxes and compared to the controls. To enhance deposition, a high number of non-gravid adult females should be used at the beginning of the test. No gravid females should be used at the initiation of a test for chemosterilant effects. For oothecal toxicity, only by bearing females should be used. The oothecae are again collected and percent eclosion compared to the control group.

B. Field trials (Experimental use permit may be required).

1. Once the appropriate dosage has been discovered in the laboratory testing, the product must be field tested to be registered. Field testing usually involves apartment houses, but food establishments or other areas with heavy infestations may also be used. The most popular methods of infestation assessment are via flashlight inspection or trapping. After the pretreatment counts are made, and application has been completed, additional counts are usually initiated on a weekly basis. Along with cockroach numbers, the degree of sanitation and any other pertinent details should be reported. Comparison treatments (i.e., a registered standard bait material) should be used as a point of reference.

III. Modifications.

1. Most testing laboratories make modifications in protocol specifications as facilities dictate. For this reason we suggest that protocols be prepared and submitted for EPA review prior to the initiation of testing. Such protocols should be submitted to the product manager for the proposed product.

(13) LaHue, D.W., and L. Bulla. 1977. Guidelines for Evaluating Efficacy of Chemical Insecticides as Protectants of Stored Grain Against Insect Pests. Bull. Ent. Soc. Amer. 23: 117-118.

Series 96: EFFICACY OF VERTEBRATE CONTROL AGENTS

§ 96-1 General considerations.

(a) Scope of vertebrate control agents. (1) A vertebrate pesticide is defined as any substance or mixture of substances intended for preventing, destroying, repelling, or mitigating any vertebrate animal which the Administrator declares to be a pest. Vertebrates include fish, amphibians, reptiles, birds, and all wild and domestic mammals. Vertebrate pesticides include oral, dermal, and inhalation toxicants; irritants; repellents (odor, taste, or tactile reaction); chemical frightening agents; anesthetizing chemicals; and reproductive inhibitors.

(b) Scope of data considerations. Toxicity data may include oral, dermal and inhalation toxicity tests for terrestrial vertebrates, or static and flow-through toxicity tests for aquatic vertebrates. These data are used to assess the appropriateness of dosage levels in subsequent efficacy testing. When conducting toxicity tests, consult references 1, 9, 10, 11, and 16 in § 96-25 for acceptable protocols. For a dose-response curve consult reference 5. Laboratory acceptance data provide information to establish bait carriers without toxicants that will be acceptable to the target animals. Laboratory efficacy data establish the preliminary effectiveness of the product under cage or pen conditions. Field efficacy data aid in determining the effectiveness of the product under actual use conditions.

(c) Pattern of considerations. (1) Specific product performance data considerations are outlined below for vertebrate pesticides in §§ 96-2 through 96-19. For each type of product (e.g., commensal rodenticides), the data considerations are addressed in the same order in which tests may be conducted: toxicity data, laboratory acceptance data, laboratory efficacy data, and field efficacy data.

(2) Under each of the kinds of data considerations stated above (toxicity, laboratory acceptance, laboratory efficacy, and field efficacy data) are the following major headings: test standards, test information, and acceptable protocols. "Test standards" prescribe the standards of an acceptable protocol. "Test information" suggests what kinds of test information should be submitted. "Acceptable protocols" cite examples of adequate protocols (when they exist) or refer the reader to those sections in § 96-30 that may be helpful in developing an acceptable protocol.

(d) Field studies. (1) Censusing methods. When conducting field studies, some means should be used in determining the number of animals before and after treatment to establish efficacy. For guidance on censusing, consult the data considerations in the specific product sections of this subdivision and in the Field Census Techniques section in § 96-30.

(2) Test and control areas. The use of test and control areas when appropriate in vertebrate control is necessary to insure that the reduction of the target pest or its damage in test areas is due to the treatment and not to other factors. Generally, both test and control areas should be of equal size and subjected to the same conditions including target animal pressures and abundance. The exact site and location of these areas will depend upon the target pests distribution, behavior and mobility. Experimental areas should be similar and include those sites when actual use is anticipated.

(3) Regional studies. Unless specified in particular sections, the number of advanced field studies necessary for each species and use pattern is five studies for each region claimed or implied on the label. These regions are the northeast, northwest, southeast, southwest, and midwest continental United States.

(e) Detoxification studies. Certain toxicants are applied in water to kill the target animals (e.g., certain pest fish). Subsequently, these aquatic areas may be restocked with more desirable species that may also be susceptible to the toxicant. Therefore, detoxification studies aid in determining how much time must elapse before restocking.

(f) Relation to other guidelines. (1) Besides the product performance data discussed in the guidelines of this subdivision, additional data may be needed when conditions such as pattern and location of use, claims made or implied, method of application, water quality, exposure period, detoxification, and species and subspecies differences indicate that the data are insufficient to answer specific questions. Registration applicants should become especially familiar with § 90-3 General considerations of this subdivision, as well as Subdivisions D - Product Chemistry, E - Hazard Evaluation: Wildlife and Aquatic Organisms, and F - Hazard Evaluation: Humans and Domestic Animals. Applicants should note that fish and wildlife toxicity data (of Subdivision E) are required for all vertebrate pesticides. Obtaining these data may require three kinds of tests: studies to determine their toxicity to nontarget organisms through primary (direct) poisoning and secondary (indirect) poisoning; reproductive studies; and field studies.

(2) In developing product performance data, testing personnel should be thoroughly familiar with the life habits and behavior of the target species and with Subdivision H - Labeling Guidelines for Pesticide Use Directions particularly §§ 100-5 and § 106-1 through 19 dealing with labeling of vertebrate control products. When designing efficacy tests for use under captive conditions, corroborative methods of field application should be kept in mind.

(3) When vertebrate pesticides are applied on or near plants or seeds, data should insure that any phytotoxicity of the formulation does not significantly reduce plant yield, plant quality, or seed germination. Such studies should be conducted on representative plants or seeds and should reflect the effects of application on plant growth or seed development through a growing cycle. The data should also reflect effects of repeat applications where such applications are recommended. The type and percentage of phyto-

toxicity should be reported. Common types of injury are leaf burning, chlorosis, and stunting. Consult Subdivision J for further data recommendations on phytotoxicity.

§ 96-2 Fish control agents.

(a) General. This section deals with efficacy data and test procedures for toxicants and repellents that claim to control fish. Target animals include lamprey, squawfish, and carp. Submission of efficacy data is waived for this section.

(b) Acute toxicity studies. Submit the results of static jar or flow-through LC_{50} tests on the active ingredient for each species and life stage (egg, larva, adult) claimed to be controlled by a fish toxicant or fish repellent. The purpose of these studies is to assess the toxicity of the active ingredients to the target species.

(1) Test standards. Refer to reference 1 of § 96-25 for test standards. To evaluate the effect of water quality on toxicity of active ingredients, for each major species to be controlled, LC_{50} tests should be conducted at: 4 water hardnesses (e.g., 11, 44, 170, 300 ppm); 4 temperatures (e.g., 7, 12, 17, 22°C); and 4 pH levels (e.g., 6.5, 7.5, 8.5, 9.5). Refer to reference 2 of § 96-25 for a discussion of selected water quality factors.

(2) Test information. Indicate the species and origin of test fish. Describe acclimation of fish, laboratory conditions (e.g., temperature, light, water quality, pH, hardness, salinity), and changes in behavior during the test that might affect efficacy. For example, were the fish repelled by a toxicant or killed by a repellent? Submit a dose response curve with the computation of the slope and LC50 with 95% confidence limits for each environmental factor evaluated (e.g., ranges on temperatures, water hardness, pH levels). For a complete list of test information, refer to page 47 of reference 1 in § 96-25.

(c) Laboratory efficacy studies. Submit the results of static jar or flow-through LC99 toxicity tests for each species and life stage (egg, larva, adult) to be controlled by the formulation. The purpose of these studies is to assess the laboratory efficacy of the formulation and to interpret the results of subsequent field tests.

(1) Test standards: Conduct LC99 tests, evaluating the same water qualities required under the LC50 test described in (b)(2) of this section.

(2) Test information. Submit the same test information requested under LC_{50} toxicity test described in (b)(3) of this section on the formulations. Indicate the chemical composition of the formulation tested.

(d) Detoxification studies. Submit the results of LC50 detoxification tests for each species claimed to be controlled. The purpose of these studies is to determine the detoxification rate of formulation.

(1) Test standards. Consult references 1, 3, and 4 of § 96-25 for detoxification test standards. Terminate tests when the biological activity

of the product has been reduced by 50% (half-life) for each major species to be controlled. Conduct tests at: 2 water hardnesses (e.g., 11 and 300 ppm); 2 temperatures (e.g., 7 and 22°C); and two pH levels (e.g., 6.5 and 9.5).

(2) Test information. Submit the same test information as under the LC_{50} toxicity test described in paragraph (b)(4) of this section. Indicate the chemical composition of the formulations, the environmental conditions, and the time interval (half-life) when the formulation has degraded to the point that a concentration of chemical that would kill 50% of the test organisms initially will now kill only 25% of the target organisms.

(3) Acceptable protocols. For conducting LC50 (static jar or flow-through tests) and for a complete list of information, refer to references 1, 3, and 4 in § 96-25. For presenting a dose response curve, consult reference 5 in § 96-25.

(e) Preliminary field efficacy studies. Submit the results of preliminary field studies conducted in small aquatic areas under natural conditions for animals claimed to be controlled. The purpose of these studies is to assess the field efficacy of formulations under semi-laboratory and natural field conditions.

(1) Test standards. Use the target species. Conduct studies in small water areas (plastic swimming pool, raceway, pond, or small stream). Natural breeding populations of target and non-target organisms are desirable. Hatchery or wild strains are acceptable. Test each formulation and dosage level that is to be tested in the field. Monitor each group's behavior frequently during the test to evaluate any unusual animal responses that might affect efficacy. See Exhibit 1 in § 96-30 for acceptable test standards, test information, and acceptable protocols.

(f) Advanced field efficacy studies. Submit the results of field efficacy tests for each species and life stage (egg, larva, adult) claimed to be controlled by a fish toxicant or fish repellent. The purpose of these studies is to assess the field efficacy of the product under actual use conditions.

(1) Test standards. (i) Number of regional studies. For each region, water type (lake, pond, stream, marsh), and species claimed or implied, submit the results from five study areas. All ranges of pH, water hardness, organic matter, temperature, and depth claimed or implied on the label should be supported by adequate field data under those environmental conditions. Minimum data considerations and the methodology to be used are similar to those in Exhibit 1 in § 96-30.

(ii) Pre-treatment and post-treatment population censusing. The density and distribution of the target fish before and after treatment should be determined by appropriate methods. Adequate statistical analysis of the data should be provided. In the case of repellents the censusing should be complete enough in the area surrounding the test area to show the movement of fish from the treated area.

(iii) Site location and selection. The test and control areas selected in each region should be representative of each of the major environmental areas in the region where the toxicant or repellent may be used (e.g., pond, lake, stream, marsh).

(2) Test information. For each test, indicate the species controlled, formulation, location of the test and control areas, and method, rate, and frequency of application. Provide a map showing location and dimensions (area and depth) of test areas. Provide all raw and summary data used to evaluate effectiveness. Include data on target and nontarget organisms and the method and rationale of selecting experimental areas. The applicant is advised to refer to Subdivision E of this part for details on evaluating nontarget effects on wildlife and aquatic organisms. Provide climatological data for study areas during the test period. Describe the statistical methods used.

(3) Acceptable protocols. Refer to Exhibit 1 of § 96-30 and the literature cited there to develop an acceptable protocol incorporating the test standards above. Prior consultation with the Agency on such protocols to be employed is strongly recommended.

§ 96-3 Aquatic amphibian control agents.

(a) General. This section gives information on efficacy data and test procedures for toxicants and repellents that claim to control aquatic stages of amphibians. Target animals include frogs, toads, salamanders, and mudpuppies. Submission of efficacy data is waived for this section.

(b) Acute and subacute toxicity studies. Submit the results of LC50 and LC99 toxicity studies for each species claimed to be controlled by an amphibian toxicant. The purpose of these studies is to assess the appropriateness of dosage levels to be used in subsequent efficacy tests.

(1) Test standards. Use the target species. Cultured animals of the target species are acceptable. Use at least 10 animals for each dosage level and formulation. Cage animals individually if fighting or cannibalism occurs. Observe any changes in behavior that may affect efficacy and gross pathological changes found at post mortem. To evaluate the effect of water quality on efficacy, LC50 tests should be conducted at 4 water hardnesses, 4 temperatures, and 4 pH levels. (Consult reference 1 in § 96-25.) Also, see § 96-2(b)(1) of this subdivision.

(2) Test information. For test information, see § 96-2(b)(2) of this subdivision.

(c) Laboratory efficacy studies for amphibian toxicants. Submit the results of the following laboratory efficacy test for each species claimed to be controlled with a toxicant. The purpose of these studies is to assess the appropriateness of dosage levels to be used in subsequent efficacy tests.

(1) Test standards. Use the target species. Laboratory or cultured strains are acceptable. Use at least 10 animals for each species and dosage level. Group animals in lots of 10 if fighting or cannibalism does not occur and if group caging of animals has been shown not to affect laboratory evaluations. If fighting or cannibalism occurs, cage and test animals separately. Test each formulation that may be tested in the field. Monitor each group's behavior frequently to evaluate any unusual animal responses that might affect efficacy.

(2) Test information. For a complete list of test information, see § 96-2(b)(2) of this subdivision.

(3) Acceptable protocols. For acceptable protocols, refer to § 96-2 (b)(3) of this subdivision.

(d) Laboratory efficacy studies for amphibian repellents. Submit the results of laboratory efficacy tests for each species claimed to be controlled with a repellent. The purpose of these studies is to assess the laboratory efficacy of formulations and to interpret results of subsequent field tests.

(1) Test standards. Use the target species. Laboratory or cultured strains are acceptable. Use at least 10 animals for each species and dosage level. Group animals in lots of 10 if fighting or cannibalism does not occur and if group caging of animals has been shown not to affect laboratory evaluations. If fighting or cannibalism occurs, cage and test animals separately. Test each formulation that may be tested in the field. Observe any changes in behavior that may affect efficacy. Replicate tests three times for all species and environmental combinations. Test the target organisms in an environment (e.g., pond, artificial or natural stream) where the organism is exposed to various concentrations of the repellent. Consult references 6 and 7 of § 96-25 for additional information.

(2) Test information. Indicate the species and origin of test animals. Describe the test apparatus and introduction of test animals. Describe the acclimation of the target species to the test situation prior to the introduction of the repellent. Provide a detailed description of the changes in animal behavior and a summary of the results of the test. Present an EC50 dose response curve for each test.

(3) Acceptable protocols. For information on recommended test environments, refer to references 6 and 7 in § 96-25. References cited in §§ 95-25 and 30 may be helpful in developing an acceptable protocol incorporating the test standards described in paragraph (d)(1) of this section. At this time, no standard protocols for evaluating amphibian repellents are available.

(e) Detoxification studies. Submit the results of LC50 detoxification tests for each species claimed to be controlled. The purpose of these studies is to determine the detoxification rate of formulations. For test standards, test information, and acceptable protocols, refer to § 93-2(d) of this subdivision.

(f) Field efficacy studies. Submit the results of field efficacy tests for each species claimed to be controlled with an amphibian toxicant or repellent. The purpose of these studies is to assess the field efficacy of the product under actual use conditions.

(1) Test standards. For test standards, see § 96-2(f)(1) of this subdivision.

(2) Test information. For information, see § 96-2 (f)(2) of this subdivision.

(3) Acceptable protocols. Refer to the literature cited in § 96-30 to develop acceptable protocol incorporating the test standards described in paragraph (f)(1) of this section. Those methods and references referring to fish toxicants may, with minor revisions, be used for amphibian toxicants and repellents.

§ 96-4 Terrestrial amphibian and reptilian control agents.

(a) General. This section provides efficacy data and test procedures for toxicants and repellents that claim to control terrestrial amphibians and reptiles. Target animals include frogs, toads, salmanders, mudpuppies, snakes, lizards, and turtles. Submission of efficacy data is waived for this section.

(b) Acute and subacute toxicity studies. Submit the results of toxicity studies for each species claimed to be controlled by an amphibian or reptilian toxicant. Acute oral LD50 and subacute LD50 tests are needed for oral toxicants. Dermal LD50 tests are needed for contact toxicants. The purpose of these studies is to assess the appropriateness of dosage levels used in subsequent testing.

(1) Test standards. Use the target species. Cultured animals of the target species are acceptable. Cage test animals individually. Use at least 10 animals for each dosage level. Observe changes in behavior that may affect efficacy. Consult standard references on holding conditions for amphibians and reptiles (see reference 8 of § 96-25) and on conducting toxicity tests (See references 8, 9, and 10).

(2) Test information. Indicate the species and origin of test animals. Describe acclimation of animals, laboratory and test conditions (e.g., light, temperature, humidity, cage size, cage type), changes in behavior during the test that may affect efficacy, and gross pathological changes found at post mortem. Provide basic toxicity data for all target animals and dose levels used. Submit a dose response curve with computation of the slope, and LD50 within 95% confidence limits for all species tested. (See reference 5 of 96-25).

(c) Laboratory efficacy studies. Submit the results of laboratory efficacy tests for each species claimed. The purpose of these studies is to assess the laboratory efficacy of the product formulations.

(1) Test standards. Use the target species. Cultured animals of the target species are acceptable. Use at least 10 animals for each species and dosage level. Use at least 10 animals for controls. The exposure period for an acute toxicant should be three days. The exposure period for a multiple-dose toxicant or repellent should be fifteen days. Evaluate the pesticide under a variety of environmental conditions.

(2) Test information. Indicate the species and origin of test animals used. Describe acclimation of animals and laboratory test conditions (e.g., temperature, humidity, light, cage size, and cage type). Submit raw and summary data showing the response of the animals to the pesticides. Submit dose response curve with computation of the slope and appropriate end points (i.e., LD50's, LD100's, EC50's, and EC100's) with 95% confidence limits for all species tested. (See reference 5 of § 96-25.)

(d) Field efficacy studies. Submit the results of field efficacy studies for each species claimed to be controlled. The purpose of these studies is to assess the field efficacy of the product under actual use conditions.

(1) Test standards. (i) Number of regional studies. The density and distribution of the target animals before and after treatment should be determined by appropriate methods. Adequate statistical analysis of the data should be provided.

(ii) Pre-treatment and post-treatment population censusing. The density and distribution of the target animals before and after treatment should be determined by appropriate methods. Adequate statistical analysis of the data should be provided.

(iii) Site selection. Test sites should be selected that permit evaluation of efficacy without significant movement of animals in or out of the census area. For example, tadpoles cannot be evaluated adequately during the metamorphosis into frogs or toads. Also, snakes cannot be evaluated when they are moving in or out of denning areas.

(iv) Environmental factors. Conduct tests under those environmental conditions (e.g., humidity, precipitation, temperature, wind) and application rates, frequencies, and methods anticipated under actual use conditions.

(2) Test information. Indicate the formulation for each study, location of sites, application method, and number of applications used. Provide a map with dimensions locating all control and test sites. Indicate the method used to select each site. Provide all raw and summary data used to evaluate efficacy. Include data on target and nontarget organisms. Provide climatological data for the study period.

(3) Acceptable protocols. References cited in § 96-30 may be helpful in developing an acceptable protocol incorporating the test standards described in paragraph (d)(1) of this section.

§ 96-5 Avian toxicants.

(a) General. This section gives efficacy and test procedures for avian toxicants. These products are applied as baits in and around buildings and in feed lots, and are applied as liquids or gels in or on perches and around buildings. Target species include the herring gull, English sparrow, house finch, pigeon, starling, brown-headed cowbird, red-winged blackbird, rusty blackbird, and common grackle. Submission of efficacy data is waived for this section.

(b) Acute and subacute toxicity studies. For a single-dose toxicant, submit the results of an acute oral LD50 test and a subacute (5-day) oral LD50 test on the target species. For a single-dose liquid or gel, also submit an acute dermal LD50 test. For a multiple-dose liquid or gel, also submit a subacute dermal LD50 test. The purpose of these studies is to assess the appropriateness of dosage levels used in subsequent testing.

(1) Test standards. Use the target species. Laboratory strains of the target species are acceptable. Use at least 10 animals for each dosage level for each sex. Cage test animals individually. Observe any changes in behavior that may affect efficacy (e.g., subject quickly becomes agitated). Observe gross pathological changes found at post mortem (e.g., hemorrhaging, edema, ulcers, discoloring). For detailed test standards, consult standard references on the subject. (See references 9, 10, and 11 of § 96-25.)

(2) Test information. Indicate the species and origin of test birds. Describe acclimation of animals, laboratory conditions (e.g., temperature, humidity, light, cage size), changes in behavior during the test that may affect efficacy, and gross pathological changes found at post mortem. Separate the mortality data by sex for each dosage level. Submit a dose response curve for each sex with the computation of the slope and LD50 with 95% confidence limits.

(c) Laboratory acceptance studies. Submit the results of laboratory acceptance studies for each species claimed to be controlled with an avian toxic bait. The purpose of these studies is to assess the appropriateness of bait carriers used in subsequent testing.

(1) Test standards. Use the target species. Laboratory strains of the target species are acceptable. Use six adult males, six adult females, and six juveniles of either sex for each species. Cage test birds individually. Provide a free choice between the habituated ration and the candidate carrier. Continue recording daily food consumption until the percentage of each food eaten stablizes. Conduct laboratory acceptance studies with all candidate carriers. Do not fast animals prior to any feeding tests. Conduct tests during regular feeding hours.

(2) Test information. Indicate the species and origin of the test animals. Describe acclimation of animals and laboratory conditions. Provide daily food consumption data for each animal. Provide summary data by sex and age, including the quantity and percentage of each diet consumed.

(3) Acceptable protocols. For conducting laboratory acceptance studies, refer to Section 8 of reference 12 in § 96-25. References cited in § 96-30 may be helpful in developing an acceptable protocol incorporating the standards described in paragraph (c)(2) of this section.

(d) Laboratory efficacy studies. Submit the results of laboratory efficacy studies for each formulation and species claimed to be controlled with an avian toxicant. The purpose of these studies is to assess the laboratory efficacy of formulations that will be tested in the field.

(1) Test standards. Use the target species. Laboratory strains of the target species are acceptable. Use six adult males, six adult females, and six juveniles of either sex for each species and formulation. Birds should be tested individually in pens where they can fly freely. Provide a free choice between the habituated ration and the candidate carrier for toxic baits, and between treated and untreated perches for liquids and gels. Test each formulation that may be evaluated in the field. Monitor each bird's behavior periodically after it is exposed to the toxicant.

(2) Test information required. Indicate the species and origin of test animals. Describe the introduction of test animals into pens, and describe pen conditions. Indicate the time and date of introduction, a description and time of all relevant behavior related to product efficacy, and the time of death for each animal. Indicate the statistical methods used to evaluate data. Provide summary data by sex, age, and species. Submit a chemical analysis of the test ration for the percentage of active ingredient. (Consult references 13 and 14 in § 96-25.

(3) Suggested performance standard. The level of efficacy that should be attained for an acceptable test has not been established. However, a 90% or greater mortality is suggested.

(4) Acceptable protocols. For conducting laboratory efficacy studies, refer to Section 5 of reference 12 of § 96-25. For existing methods of chemical analysis, see references 13 and 14 of § 96-25.

(e) Field efficacy studies. Submit the results of field efficacy tests for each species claimed to be controlled with an avian toxicant. The purpose of these studies is to assess the field efficacy of the product under actual use conditions.

(1) Test standards. (i) Preliminary field testing. Monitor preliminary field testing very closely in a well-delineated area to uncover any unforseen problems with product efficacy or nontarget hazard before extensive regional testing is initiated.

(ii) Number of regional studies. For each site, formulation, species, method of application, major region, and species claimed or implied on the label, submit five field efficacy studies.

(iii) Pre-treatment and post-treatment population censusing. Direct censusing is always required and involves visual counts of birds are made at periodic intervals (e.g., early morning) for several days in order to obtain a relative index of bird pressure. Both target and non-target bird species should be censused. In indirect censusing, random samples of crops are taken and evaluated for percentage of damage. Indirect censusing is always needed when the chemical is intended to protect a crop. For these situations, yield comparisons (projected or actual) and cost/benefit analyses can also be made. Make periodic counts of dead target and non-target animals. Indicate the species and sex.

(iv) Site selection. It is important that all experimental groups have comparable and adequate damage or bird pressure. Otherwise, the study will be biased. An experimental area should not be included either as a test or control unless it is experiencing a predetermined amount of damage or pressure. Equal numbers of test and control areas can be selected in two ways: at random, or by assigning areas with comparable damage to the test and control groups.

(v) Rate, frequency, and method of application. In order to provide adequate use directions on the label, efficacy testing should include trials at different rates (e.g., 1 lb/acre or 4 oz. per placement); frequencies (e.g., 1 application per week or month); and method of application (e.g., ground, hand or aerial). If a claim is made that a product is effective for a particular time period, then the test design should reflect studies covering that period of time.

(vi) Effect of environmental factors and managerial practices. Studies should be conducted under the environmental factors anticipated under actual use conditions. Environmental factors (such as precipitation, temperature, light, humidity, and wind), as well as managerial practices (such as sanitation, timing of planting, and location of crops), may have an impact on product effectiveness. These variables should be taken into account in the experimental test design.

(vii) Suggested performance standard. Although the level of efficacy that should be attained has not been established, a 70% reduction in the target pest numbers, activity, or damage is suggested.

(2) Test information. Indicate the locations, formulations, site, method, frequency, and number of applications for each study. Provide a map of test and control areas, including their dimensions. Provide all raw and summary data used to evaluate effectiveness. Include information on target and non-target species. Indicate method and rationale of selecting experimental areas and of assigning them to test and control groups. Provide climatological data for the study period. Include all information on the statistical methods used.

(3) Acceptable protocols. For conducting field efficacy studies, consult Section 9 of reference 12 in § 96-25. References cited in § 96-30 may be helpful in developing an acceptable protocol incorporating the test standards above.

§ 96-6 Avian repellents.

(a) General. This section furnishes information on efficacy data and test procedures for avian repellents applied to seed. Target species include the starling, red-winged blackbird, common grackle, pheasant, Oregon junco, and Canada jay. Submission of efficacy data is waived for this section.

(b) Preliminary acute and subacute toxicity studies. Submit the results of toxicity studies for each species claimed to be controlled with an avian repellent applied to seed. These studies shall include an acute oral LD50 test and a subacute (5-day) oral LD50 test for the bobwhite quail. The purpose of these studies is to assess the possibility of hazard to the target species.

(1) Test standards. Use the bobwhite quail. Laboratory strains are acceptable. Use at least 10 birds for each dosage level for each sex. Cage test birds individually. Observe any changes in behavior that may affect efficacy. Consult standard references on the subject. (See references 9 and 11 in § 96-25.)

(2) Test information. Indicate species and origin of test birds. Describe acclimation of birds, laboratory conditions (e.g., temperature, humidity, light, cage size), changes in behavior during the test that may affect efficacy, and gross pathological changes found at post mortem. Separate the mortality data by sex for each dosage level. Submit a dose response curve for each sex with the computation of the slope and LD50 with 95% confidence limits. (See reference 5 in § 96-25.)

(c) Advanced acute and subacute toxicity studies. If either test listed above yields an LD50 of 1000 mg/kg or less, conduct tests using the target bird claimed to be controlled by an avian repellent. The purpose of these studies is to assess the toxicity and possibility of hazard to target species.

(1) Test standards. Use the target species or genus. Laboratory strains are acceptable. Use at least 10 birds for each dosage level for each each sex. Cage birds individually. Observe any changes in behavior that may affect efficacy. Observe gross pathological changes found at post mortem (e.g., hemorrhaging, edema, ulcers, discoloring). Consult standard references on the subject. For detailed test standards, consult references 9 and 11 in § 96-25.

(2) Test information. Indicate species and origin of the test birds. Describe acclimation of birds, laboratory conditions (e.g., temperature, humidity, light, cage size), changes in behavior during the test that may affect efficacy, and gross pathological changes found at post mortem. Separate the mortality data by sex for each dosage level. Submit a dose response curve for each sex with the computation of slope and LD50 with 95% confidence limits.

(d) Laboratory efficacy studies. Submit the results of a laboratory efficacy test for each species claimed to be controlled. The purpose of these studies is to assess the laboratory efficacy of the formulation.

(1) Test standards. Use the target species. Use animals that are laboratory-reared or captured from wild environments. Maintain wild-caught birds destined for testing in communal cages for a minimum of 2 weeks to acclimate them to captivity. Maintain laboratory-reared and acclimated wild-caught birds for 3 to 7 days in the type of pen or cage used for the test. Use test cages or pens that allow free movement of the test bird and free access to food and water. Use a single, readily available, normal or preferred food of the test species as the habituated

diet. At least 20% of the diet should comprise the food item to be tested. Insure that the diet and general condition have stabilized for one week prior to the test. Maintain stable environmental conditions in the laboratory. Do not fast test birds prior to testing. For at least two days prior to the test, birds are to be individually caged and offered a weighed quantity of food in excess of daily requirements in two identical food containers. Their locations should be switched daily. Birds consuming less than 60% of the mean food consumption of all pretest birds shall not be included in further testing. Use a minimum of 5 adult males, 5 adult females, and 5 juveniles of either sex for each treatment and species. Transfer birds to clean individual cages. Provide each bird with two clean food containers, one with treated food and the other untreated food. Continue the test for at least four days. Record daily food consumption for each diet and for each bird. Reverse position of food containers daily. Use 3 to 5 treatments to establish the ED50. Record death, sickness, and unusual behavioral changes in test birds according to age, sex, and species.

(2) Suggested performance standard. A bird is considered to be repelled if more than 75% of the total amount of food eaten during the 4 days of the test is untreated.

(3) Test information. Report date test was commenced. Identify the species and origin of test birds. Describe acclimation of the birds and laboratory conditions (e.g., light, temperature, humidity, cage size). Submit the following information for each bird: sex, age, initial and final weight. Indicate the composition and concentration of each formulation and how the repellent was applied to the food. Submit the following information for the food particles: the type, source, size, and average weight. Report daily and summary treated and untreated food consumption data by sex, age, and species. Provide information according to sex, age, and species on deaths, sickness, or unusual behavioral changes. Submit a dose response curve for each sex and age with the computation of the slope and ED50 with 95% confidence limits. (See reference 5 in § 96-25).

(4) Acceptable protocols. For presenting a dose response curve, refer to reference 5 in § 96-25. For conducting laboratory efficacy studies, consult reference 15 in § 96-25.

(e) Field efficacy studies. Submit the results of field efficacy tests for each species claimed to be controlled. The purpose of these studies is to assess the field efficacy of the product under actual use conditions.

(ii) Pre-treatment and post-treatment censusing. Direct and indirect censusing in treated and untreated areas should be undertaken. In direct censusing, the species (target and non-target) are counted with optical aids for 3 or more days at specific times during the day (e.g., 7:00 to 7:30 AM). In indirect censusing, the number of seeds removed per unit area is counted until seeds are no longer subject to bird pressure.

(iii) Rate, frequency, and method of application. In order to provide adequate use directions on the label, efficacy testing should include trials at different rates (e.g., 10 oz. product per 100 lbs. of seed), frequencies (e.g., 1 application per week or month), and methods of application (e.g., ground, hand, or aerial). If a claim is made that a product is effective for a particular time period, then the test design should reflect studies covering that period of time.

(iv) Effect of environmental factors and managerial practices. Studies should be conducted with the environmental factors anticipated under actual use conditions. Environmental factors (such as precipitation, temperature, light, humidity, and wind), as well as managerial practices (such as sanitation, and timing and location of planting), may have an impact on product effectiveness. These variables should be taken into account in the experimental design.

(2) Suggested performance standard. The level of efficacy that should be attained for an acceptable test has not been established. However, if 70% fewer seeds treated with the product are removed by the target population, in comparison to untreated seeds, such a level of efficacy would be is suggested.

(3) Test information. Indicate the target species. Include a map with dimensions of all treated and untreated study areas. Indicate how experimental areas were selected and how they were assigned to treatments and controls. Report the method and frequency of application, the formulation, dosage rates, temperature, precipitation, and the soil moisture. Include all raw and summary data upon which direct and indirect censusing were made. Record the species and sex of all dead animals (target and non-target) found in treated and control areas. Describe all statistical methods used to evaluate efficacy.

(4) Acceptable protocols. References cited in § 96-30 may be helpful in developing an acceptable protocol incorporating the test standards described in paragraph (e)(2) of this section.

§ 96-7 Avian frightening agents.

(a) General. This section deals with efficacy data and test procedures for avian frightening agents. This guidance refers to those agents applied as baits in and around buildings, in crops, in dumps, and at airports. Target species include the herring gull, English sparrow, pigeon, starling, brown-headed cowbird, red-winged blackbird, and common grackle. Submission of efficacy data is waived for this section.

(b) Acute and subacute toxicity studies. Submit the results of an acute oral LD50 test and subacute (5-day) oral LD50 test for each species claimed to be controlled with an avian frightening agent. The purpose of these studies is to assess the appropriateness of dosage levels used in subsequent testing.

(1) Test standards. Use the target species. Laboratory strains of the target species are acceptable. Use at least 10 animals for each dosage level for each sex. Cage test animals individually. Observe any changes in behavior that may affect efficacy. Observe gross pathological changes found at post mortem (e.g., hemorrhaging, edema, ulcers, discoloring). Check standard references on the subject for detailed test standards. (Consult references 9 and 11 in § 96-25.)

(2) Test information. Indicate the species and origin of test animals. Describe acclimation of animals, laboratory conditions (e.g., temperature, humidity, light, cage size), changes in behavior during the test that may affect efficacy, and gross pathological changes

found at post mortem. Separate the mortality data by sex for each dosage level. Indicate the oral LD50 of the product for each sex. Submit a dose response curve with the computation of the slope and LD50 with 95% confidence limits. (See reference 5 in § 96-25.)

(c) Laboratory acceptance studies. Submit the results of laboratory acceptance studies for each species claimed to be controlled with an avian frightening agent. The purpose of these studies is to assess the appropriateness of bait carriers used in subsequent testing.

(1) Test standards. Use the target species. Laboratory strains of the target species are acceptable. Use six adult males, six adult females, and six juveniles of either sex for each species. Cage test animals individually. Provide a free choice between the standard ration the animals are accustomed to eating and the candidate carrier. Continue recording daily food consumption until the percentage of each food eaten stabilizes. Conduct laboratory acceptance studies with all candidate carriers. Do not fast the animals prior to any feeding hours. Conduct tests during regular feeding hours. Consult reference 12 in § 96-25 for additional information.

(2) Test information. Indicate the species and origin of test animals. Describe acclimation of animals and laboratory conditions. Provide daily food consumption data for each animal. Report summary data by sex and age, including the quantity and percentage of each diet consumed.

(3) Acceptable protocols. For information on laboratory acceptance studies, review Section 8 of reference 12 in § 96-25. References cited in § 96-25 on avian frightening agents may be helpful in developing an acceptable protocol incorporating the test standards described in paragraph (c)(2) of this section.

(d) Laboratory efficacy studies. Submit the results of laboratory efficacy studies for each species claimed to be controlled with an avian frightening agent. The purpose of these studies is to assess the laboratory efficacy of formulations that will be tested in the field.

(1) Test standards. Use the target species. Laboratory strains of the target species are acceptable. Use six adult males, six adult females, and six juveniles of either sex for each species and formulaation. Test animals individually in pens where the birds can fly from from perch to perch. Test each formulation that may be tested in the field. Monitor each bird's behavior continuously after it is introduced into the pen with the candidate formulation. Consult reference 12 in § 96-25 for additional information.

(2) Test information. Indicate the species and origin of test animals. Describe the introduction of test animals into pens and pen conditions. Provide the time of introduction, a description and time of all relevant behavior related to product efficacy, and time of death for each animal. Report summary data by sex, age, and species. Submit a chemical analysis of the test ration for the percentage of active ingredient. (See references 13 and 14 in § 96-25.)

(3) Acceptable protocols. For information on laboratory efficacy studies, refer to Section 5 of reference 12 in § 96-25. For methods of chemical analysis, consult references 13 and 14 in § 96-25.

(e) Field efficacy studies. Submit the results of field efficacy tests for each species claimed to be controlled with an avian frightening agent. The purpose of these studies is to assess the field efficacy of the product under actual use conditions.

(1) Test standards. (i) Number of regional studies. Submit 5 field efficacy studies for each site (e.g., in and around buildings, in sunflowers, in field corn, in sweet corn, in dumps, at airports), formulation, method of application, major region, and species claimed or implied to be controlled.

(ii) Pre-treatment and post-treatment population censusing. Two basic methods of censusing are used, one of which is direct and the other is indirect. In direct censusing, visual counts of birds are made at periodic intervals, (e.g., early morning) for several days in order to obtain a relative index of bird pressure. Both target and non-target bird species should be censused. Direct censusing is always recommended. In indirect censusing, random samples of crops are made and evaluated for percentage of damage. For some sites such as sunflowers, sweet corn, and field corn, the U.S. Department of the Interior has developed standard methods of direct and indirect censusing. Indirect censusing is always recommended when the chemical is intended to protect a crop. For these situations, yield comparisons (projected or actual) and cost/benefit analyses can also be made.

(iii) Site selection. All experimental groups should have comparable and adequate damage or bird pressure. Otherwise, the study will be biased. A field should not be included either as a test or control area unless it is experiencing a predetermined amount of damage or pressure. Equal numbers of test and control areas can be selected in two ways: at random, or by assigning areas with comparable damage to the test and control areas.

(iv) Rate, frequency, and method of application. To provide adequate use directions on the label, the tester should design efficacy testing to include trials at different rates (e.g., 1 lb. per acre or 4 oz. per placement), frequencies (e.g., 1 application per week or month), and methods of application (e.g., ground, hand, or aerial). If a claim is made that a product is effective for a particular time period, then the test design should reflect studies covering that period of time.

(v) Effect of environmental factors and managerial practices. Studies should be conducted under environmental factors anticipated under actual use conditions. Environmental factors (such as precipitation, temperature, light, humidity, and wind), as well as managerial practices (such as sanitation, timing of planting, and location of crops), may have an impact on product effectiveness. These variables should be taken into account in the experimental test design.

(vi) Initial field testing. Monitor initial field testing very closely in a well delineated area to uncover any unforeseen problems with product efficacy or nontarget hazard before extensive regional testing is initiated. Consult reference 12 in § 96-25 for additional information.

(vii) Suggested performance standard. Although the level of efficacy that should be maintained has not been established, a 70% reduction in activity or damages due to use of the product is suggested.

(2) Test information. For each study, indicate the formulation, location and size of test area(s), and the method, number, rate, and frequency of applications. Provide a map of test and control areas, including their dimensions. Report all raw and summary data used to evaluate effectiveness. Include information on target and non-target species. Indicate the method and rationale of selecting experimental areas and of assigning them to test or control groups. Report type and degree of damage being experienced by test and control areas. Provide climatological data for the study period. Describe all statistical methods used.

(3) Acceptable protocols. For information on field efficacy studies, see Section 9 of reference 12 in § 96-25. References cited in § 96-30 may be helpful in developing an acceptable protocol incorporating the test standards described in paragraph (e)(2) of this section.

§ 96-8 Mole toxicants.

(a) General. This section presents efficacy data and test procedures for mole toxicants applied as baits and fumigants to underground tunnels. Target species include the eastern mole, Townsend mole, starnose mole, and California mole. Submission of efficacy data is waived for this section.

(b) Acute and subacute toxicity studies. Submit the results of toxicity studies for each genus of mole claimed to be controlled by a toxicant applied as bait or toxicant. For a single-dose bait, submit an acute oral LD50 test. For a multiple-dose bait, submit an acute oral LD50 test and a subacute (5-day) oral LD50 test. For a fumigant, submit an acute inhalation LC50 test. The purpose of these studies is to assess the appropriateness of dosage levels used in subsequent testing.

(1) Test standards. Use the target genera. Use animals that are laboratory-reared or captured from wild environments. Use at least 10 animals for each dosage level. If external sexing is not possible, determine sex at post mortem. Cage test animals individually. Observe any changes in behavior that may affect efficacy. Observe gross pathological changes found at post mortem (e.g., hemorrhaging, edema, ulcers, discoloring). For detailed test standards, consult references 9 and 16 in § 96-25.

(2) Test information. Indicate the species and origin of test animals. Describe acclimation of animals, laboratory conditions (e.g., temperature, humidity, light, cage size), and gross pathological changes found at post mortem. Separate mortality data for each dosage level. Submit a dose response curve for each species claimed or implied with computation of the slope and LD50 or LC50 with 95% confidence limits. (See reference 5 in § 96-25.)

(c) Laboratory acceptance studies. Submit the results of laboratory acceptance studies for each genus claimed to be controlled with a toxic bait. The purpose of these studies is to assess the appropriateness of bait carriers used in subsequent testing.

(1) Test standards. Use the wild target genus. Use at least 10 adult moles. If external sexing is not possible, determine sex at post mortem. Cage test animals individually. Provide a free choice between the candidate bait carrier and the habituated diet. Continue recording daily food consumption until the percentage of food eaten stablizes. Conduct laboratory acceptance studies with all candidate carriers that may be tested in the field.

(2) Test information. Indicate the species and origin of test animals. Describe acclimation of animals and laboratory conditions. Submit individual and summary data on all moles, including the quantity and percentage of each diet consumed.

(d) Laboratory efficacy studies. Submit the results of a laboratory efficacy test for each genus claimed to be controlled. The purpose of these studies is to assess the laboratory efficacy of the formulation.

(1) Test standards. Use the wild target genus. Use at least 10 adult moles for each test. If external sexing is not possible, determine sex at post mortem. Use at least 10 adult moles for controls. Greater than 10% mortality in controls negates the test. For toxic baits, provide a free choice between the habituated ration (e.g., fish-flavored cat food) and the toxic bait. For fumigants, test animals individually in an artificial mole burrow. (See reference 17 in § 96-25.) The exposure period for a single-dose bait is 3 days. The exposure period for a multiple-dose bait is 15 days. The exposure period for a fumigant is one hour. Test all formulations that may be used in the field.

(2) Suggested performance standard. The level of efficacy that should be attained for an acceptable test has not been established. However, 90% mortality in the test group and no more than 10% mortality in the control group is suggested.

(3) Test information. Identify the species and origin of the target animals. Describe acclimation of the animals and laboratory conditions. For a fumigant, describe the method of application. For a toxic bait, provide daily treated and untreated food consumption data for each test animal and species. For each animal, indicate the time of death. Provide summary data by sex and species. Submit a chemical analysis of the test ration for the percentage of active ingredient. (See references 13 and 14 in § 96-25.)

(4) Acceptable protocols. References 17 and 18 in § 96-25 and others cited in the § 96-30 may be helpful in developing an acceptable protocol incorporating the test standards above. For methods of chemical analysis, consult references 13 and 14 in § 96-25.

(e) Field efficacy studies. Submit the results of field efficacy tests for each species claimed to be controlled. The purpose of these studies is to assess the field efficacy of the product under actual use conditions.

(1) Test standards. (i) Number of regional studies. Submit the results of three acceptable field efficacy studies for each formulation, method of application, major region, and species claimed or implied to be controlled.

(ii) Pre-treatment and post-treatment population censusing. Locate mole-infested fields, lawns, and other areas where interference by cats, dogs, or other animals is not anticipated. Using surface burrows, identify and mark burrow systems individually. A single burrow system would include all interconnecting surface burrows. In a period of five days, identify 20 active burrow systems. Punch one-inch holes in the roof of each burrow of a burrow system. Burrow systems are defined as "active" if holes have been opened and plugged at least three times within five days. Mark the locations where burrows are punched so that the same tunnels can be punched during the post-treatment phase. As each of the 20 active burrow systems is located, number them so that the closest active burrow systems have consecutive numbers.

(iii) Treatment phase. Treat the odd-numbered burrow systems but not the even numbered burrow systems. In this way, only alternate systems are treated. Treat in a consistent manner, according to the directions to be included on the product label. Maintain records on the methods of application, location and quantity of bait or fumigant, and the frequency of application.

(iv) Treated and untreated burrows. The use of treated and untreated burrows insures that the reduction in the number of the target pests in treated burrows is due to the treatment and not to other factors.

(v) Verification of the target species. Because the method of censusing is an indirect one, some live trapping before censusing should be done to verify the species of mole. Record the species of mole captured.

(vi) Suggested performance standard. The level of efficacy that should be attained for an acceptable test has not been established. However, a 70% reduction in burrowing activity of the target population due to the use of the product is suggested.

(2) Test information. Report the results of all studies, including raw and summary data. Submit information for each treated burrow system on the method and frequency of application, and the location, composition, and quantity of bait or fumigant. Provide a general map showing the numbers and approximate locations of punched tunnels, dimensions of the burrow systems, distance to the closest active burrow system, and the location of treatments. For all phases of the study, include climatological data (daily maximum and minimum temperature, and daily precipitation) from a local weather station. Submit information on mortality by species and sex for target and non-target kills in study areas. Include all figures used to calculate the percentage of efficacy.

(3) Acceptable protocols. Reference 17 in § 96-25 and others cited in § 96-30 may be helpful in developing an acceptable protocol incorporating the test standards described in paragraph (e)(2) of this section. Prior consultation with the Agency on such protocols to be employed is strongly recommended.

§ 96-9 Bat toxicants and repellents.

(a) General. This section gives efficacy guidance for bat toxicants and repellents applied indoors. Target species include the little brown bat and the big brown bat. Submission of efficacy data is waived for this section.

(b) Acute and subacute toxicity studies. Conduct acute and subacute (5-day) oral LD50 studies with target species. Inhalation and/or dermal LD50 studies may be required depending upon the nature of the active ingredient and its apparent mode of action. The purpose of these studies is to assess the appropriateness of dosage levels used in subsequent testing.

(1) Test standards. Use the target species. Use 10 adult animals of each sex for each dosage level. Cage animals individually. Describe acclimation of animals, laboratory conditions (e.g., temperature, humidity, lighting, cage size), symptoms of poisoning, and gross pathological changes.

(2) Test information required. Indicate the species tested, the location of capture, the length of time in captivity, and holding conditions. Provide weights of animals at capture, before dosing, and after death or recovery. Ten males and 10 females should be tested at each dosage level. Submit a dose response curve with the computation of the slope and LD50 with 95% confidence limits. (See reference 5 of § 96-25.) The time and date of symptom onset and the date of death (if any) should be noted for all subjects.

(c) Laboratory efficacy studies. Submit the results of laboratory efficacy studies for each target species. The purpose of these studies is to assess the laboratory efficacy of formulations that will be tested in the field.

(1) Test standards. Use the target species. Use at least ten adult males and ten adult females for each target species and each formulation. Use a control group maintained under the same conditions as test subjects but without exposure to control agent. Record weights of bats at capture and before and after test. Acclimate bats for two weeks prior to test. For maintaining bats in captivity, consult reference 19 in § 96-25. For claims of repellency, quantitative definitions are needed. Repellency may be defined as an apparent reduction of activity in a certain (treated) portion of a test enclosure. Protocols should be reviewed by the Agency before tests are conducted.

(2) Suggested performance standard. No performance standards have been established for bat products. However, 90% mortality would be adequate for toxicants and 80% reduction in activity would be adequate for repellents.

(3) Test information. For each study, indicate the formulation, type of claim, and details of capture of animals. Describe laboratory and test enclosure acclimation procedures. Report weights of animals at capture, at the onset of testing, and at the conclusion of testing. Submit a diagram of the test enclosure, details of procedures and rates of application, records of behavioral responses relevant to the claims being tested, records of symptoms of toxicity, and dates of death (if any). Provide all raw and summary data for test and control animals. For all products, the length of time between the onset of symptoms and death should be noted. Also, note the length of time that symptoms were present in any subjects that recovered from apparent poisoning.

(4) Acceptable protocols. No specific complete test protocols exist for laboratory evaluation of bat toxicants and repellents. However, references cited in § 96-30 may be helpful in developing acceptable protocols incorporating the test standards indicated in paragraph (c)(2) of this section.

(d) Field efficacy studies. Submit the results of all field efficacy tests for each species and each use pattern claimed. The purpose of these studies is to assess the field efficacy of the product under actual use conditions.

(1) Test standards. (i) Number of regional studies. For each formulation, method of application, major region, type of site, and bat species claimed to be controlled, submit the results of at least five acceptable field efficacy studies. Every field efficacy study site must use a corresponding control site.

(ii) Site. Treatments should be made in indoor sites only.

(iii) Pre-treatment and post-treatment population censusing. Control is measured in terms of estimates of population reduction (for toxicant claims) or in percent bat activity reduction (for repellency claims). Estimation procedures should be reviewed by the Agency prior to the conducting of tests. Extensive pre-treatment data should be collected to determine normal levels of occupancy of sites by bats. Procedures for censusing bats are referenced in § 96-30. Under certain conditions, direct counts may be used. Two sampling methods should be used unless it is possible for direct counts to be absolute.

(iv) Suggested performance standard. No performance standards have been established. However, 90% control due to use of the product is suggested.

(2) Test information. Indicate target species, methods of censusing, pre-and post-treatment census results, application procedures, and application rates. Report numbers of dead bats found and any non-target deaths. Describe significant changes in bat behavior, observable symptoms of toxicity, weather conditions (temperature, humidity, precipitation--especially for enclosures with leaky roofs), and any other data relevant to the assessment of efficacy.

(3) Acceptable protocols. No standard protocols exist for field evaluation of the efficacy of bat control products. However, references cited in Section 96-30 may be helpful in developing acceptable protocols incorporating the test standards described in paragraph (d)(2) of this section.

§ 96-10 Commensal rodenticides.

(a) General. This section concerns guidance regarding efficacy and test procedures for commensal rodenticides used in or around buildings. Target species are commensal rodents such as the Norway rat, roof rat, Polynesian rat, and house mouse. Commensal rodents are those that live at man's expense, usually living in his home or buildings, and sharing with man their diseases and many other undesirable traits, without contributing anything beneficial to the relationship. Submission of efficacy data is waived in this section.

(b) Acute and subacute toxicity studies. Submit the results of toxicity studies for each species claimed to be controlled with a commensal rodenticide. For single-dose rodenticides, submit an acute oral LD50 test. For multiple-dose rodenticides, submit an acute oral LD50 test and a subacute (5-day) oral LD50 test. The purpose of these studies is to test the appropriateness of dosage levels used in subsequent testing.

(1) Test standards. Use the target species. Laboratory strains of target species are acceptable. Cage test animals individually. Test both juvenile animals (within 21 days after weaning) and adult animals (over 35 days after weaning). Tests are not required for juvenile roof rats. For each sex and age group, use at least 10 animals for each dosage level. Observe changes in behavior that may affect efficacy. Observe gross pathological changes found at post mortem (e.g., hemorrhaging, edema, ulcers, discoloring). For detailed test standards, consult a standard reference on the subject. (See reference 9 in § 96-25.)

(2) Test information. Indicate species and origin of test animals. Describe acclimation of animals, laboratory conditions (e.g., temperature, humidity, light, cage size), changes in behavior during test, and gross pathological changes found at post mortem. Separate the mortality data by age and sex for each dosage level. Indicate the number dying at each dosage level for each sex and age group. Submit a dose response curve with the computation of the slope and LD50 with 95% confidence limits. (See reference 5 in § 96-25.)

(3) When the applicant or registrant is notified by the Agency that samples are necessary for confirmatory toxicity testing on rats and mice, such samples should be sent to the EPA Animal Biology Laboratories at the following address: Building 402 Agr. Res. Center-East, Beltsville, Md. 20705.

(c) Laboratory efficacy studies. Submit the results of a laboratory efficacy test for each species claimed to be controlled with a commensal rodenticide. The purpose of these studies is to assess the laboratory efficacy of the formulation.

(1) Test standards. For complete test standards and protocols, refer to Exhibits 2 through 20 in § 96-30. Use the wild target species for a new claim. Otherwise, use either the wild target species or laboratory strains of the target species. In the latter case, a roof rat claim may be supported by Norway rat data. Use at least 10 males and 10 females for each test. Use at least 10 males and 10 females for controls. A standard challenge diet is provided whenever the animals are exposed to a toxic bait. Refer to Exhibits 2 through 20 in § 96-30 for appropriate challenge diets. For a tracking powder, the animal is always provided with a non-toxic route to all sources of food and water. For a liquid bait, an alternate, nontoxic water source must be provided. The exposure period for a single-dose toxicant is 3 days. For a multiple-dose toxicant, the exposure period is 15 days. Some very toxic anticoagulant baits may be tested for only 3 days. Rodenticide baits presented to a species in an unopened place pack must have a test on the formulation itself and with the formulation in the place pack.

(2) Suggested performance standards. In an acceptable laboratory efficacy test, the product should pass the following levels of mortality and acceptance. Anticoagulant dry baits should achieve 90% mortality and 33% acceptance (the toxic bait comprises 33% of the total food consumed). Anticoagulant dry baits that are offered to the test rodent for a maximum of 3 days should achieve at least 90% mortality. Acute dry baits, liquid baits, and tracking powders should achieve 90% mortality. Red squill baits and tracking powders should achieve 75% mortality. Wax blocks and wax pellets should achieve 80% mortality and 25% acceptance. Greater than 10% mortality in controls negates the test.

(3) Test information. Indicate the species and strain of animal used, the origin of test animals, and the weights of the animals at the beginning and end of the test. Describe the laboratory conditions (e.g., temperature, humidity, light, and cage size) and acclimation of animals. Submit the raw data for daily consumption of toxic bait and challenge diet. Indicate the day of death for each animal. Provide summary data showing the mortality and the total amount and percentage of toxic bait consumed. Submit a chemical analysis of the test ration for the percentage of active ingredient. (See references 13 and 14 in § 96-25.)

(4) Acceptable protocols. Refer to Exhibits 2 through 20 in § 96-30 for acceptable protocols. For existing methods of chemical analysis, consult references 13 and 14 in § 96-25.

(5) Data submitted to support laboratory efficacy claims which are made for products recommended for use on rats and mice may be confirmed by tests conducted by the Agency's Animal Biology Laboratory when requested before registration.

(d) Field efficacy studies. Submit the results of field efficacy studies for each species claimed to be controlled with a commensal rodenticide. Data should be submitted for a new claim. If a dry bait will be marketed in more than one form (meal, pellet, or wax block), field tests should be conducted with at least one of these formulations. The purpose of these studies is to assess the field efficacy of the product under actual use situations.

(1) Test standards. (i) Indoor and outdoor studies. If an indoor use use is claimed, only the indoor studies are needed. For outdoor studies, the following types of sites are acceptable: sheds (covered, non-enclosed structures); small dumps that are not being continually reinfested; and small isolated stockyards (all placements in tamper-proof bait boxes or in rat burrows).

(ii) Number of regional studies. (A) Dry bait studies. Norway rat: 5 indoor studies (one in each of the five major regions of the country) and 2 outdoor studies (each in a different region). Roof rat: 2 indoor studies (in different regions) and 1 outdoor study. House mouse: 5 indoor studies (1 in each region) and 1 outdoor study.

(B) Tracking powder or liquid bait studies: Norway rat: 5 indoor studies (any region), and 2 outdoor studies (any region). Roof rat: 2 indoor studies (any region) and 1 outdoor study. House mouse: 5 indoor studies (any region), and 1 outdoor study.

(iii) Pre-treatment and post-treatment population censusing. For commensal rodenticides, at least two methods of pre-treatment and post-treatment censusing are needed, one of which cannot be snap trapping. However, the post-treatment census should be followed by 3 days of snap trapping. The use of snap traps is necessary to complement the two census methods as to the number of rodents present and/or to confirm the existence of the target species. Some acceptable censusing methods are live trapping, food consumption, non-toxic tracking dust, and smoked milk cartons. If live trapping is used, animals should be marked individually. If live trapping is not one of the census methods, some live trapping prior to pre-treatment censusing may be necessary to confirm the presence of the test species. If food consumption is used, there should be at least one placement inaccessible to animals but open to the air, to determine moisture uptake. Otherwise, all baits should be dried to a constant weight prior to placement and after removal. If non-toxic tracking dust or smoked milk cartons are used, some preliminary work in the laboratory will be needed to determine how long the dust or cartons have to be exposed before rats or mice begin leaving tracks. A suggested rating system is: 0 = no activity, 1 = little activity (1-3 tracks), 2 = moderate activity (4-6 tracks), and 3 = heavy activity (7 or more tracks).

(iv) Site selection. Select sites that are isolated as much as possible from areas that may serve as reservoirs of reinfestation. Treat all buildings at test sites. Every field efficacy study site should use a corresponding control site.

(3) Suggested performance standard. Acceptable studies are those that show 70% or greater reduction in the target population activity (due to use of the product) in at least two of the censusing techniques outlined above, and that show no more than one target animal snap-trapped per 10 traps set per night.

(4) Test information. Report the results of all studies. Submit a sketch of the test sites, indicating the location and approximate dimensions of all buildings. Also indicate where the baits, powder, and traps were placed. Provide all raw data used for computing population levels. Include all data on amount of toxic bait applied and consumed. Weigh, identify, and sex all animals, including non-target species found dead. Perform a post mortem on these animals to determine cause of death. Indicate the method used for marking animals.

(5) Acceptable protocols. No specific complete test protocols exist for field evaluation of commensal rodenticides. However, protocols cited in § 96-30 may be helpful in developing an acceptable protocol incorporating the test standards indicated in paragraph (d)(2) of this section.

§ 96-11 Rodenticides in orchards.

(a) General. This section furnishes information on efficacy data and test procedures for rodenticides applied as baits or ground sprays in orchards. Target species include the meadow vole and pine vole. Submission of efficacy data is waived for this section.

(b) Acute and subacute toxicity studies. Submit the results of toxicity studies for each species claimed to be controlled with a toxic bait or ground spray in an orchard. For a single-dose bait or ground spray, submit an acute oral LD50 test. For a multiple-dose bait or ground spray, also submit an acute dermal LD50 test. The purpose of these studies is to assess the appropriateness of dosage levels used in subsequent testing.

(1) Test standards. Use the wild target species. Use at least 10 animals for each dosage level for each sex. Cage test animals individually. Observe any changes in behavior that may affect efficacy. Observe gross pathological changes found at post mortem (e.g., hemorrhaging, edema, ulcers, discoloring). Consult standard references on the subject for more detailed test standards. (See references 9 and 10 in § 96-25.)

(2) Test information. Indicate the species and origin of test animals. Describe acclimation of animals, laboratory conditions (e.g., temperature, humidity, light, cage size), changes in behavior that may affect efficacy, and gross pathological changes found at post mortem. Separate the mortality data by sex for each dosage level. Submit a dose response curve for each sex with the computation of the slope and LD50 with 95% confidence limits. (See reference 5 in § 96-25.)

(c) Laboratory acceptance studies. Submit the results of laboratory acceptance studies for each species claimed to be controlled with a toxic bait. The purpose of these studies is to assess the appropriateness of bait carriers used in subsequent testing.

(1) Test standards. Use the wild target species. Use six adult males, six adult females, and six juveniles of either sex for each target species. Cage test animals individually. Provide a free choice between the rat and mouse challenge diet and the candidate carrier. Continue recording daily food consumption until the percentage of each food eaten stabilizes. Conduct laboratory acceptance studies with all candidate carriers. Do not fast the animals prior to any feeding tests. Conduct tests during regular feeding hours.

(2) Test information. Indicate the species and origin of test animals. Describe acclimation of animals, and laboratory conditions. Submit daily food consumption data for each animal. Provide summary data by sex and age, including the quantity and percentage of each diet consumed.

(3) Acceptable protocols. References cited in § 96-30 may be helpful in developing an acceptable protocol incorporating the test standards described in paragraph (c)(2) of this section.

(d) Laboratory efficacy studies. Submit the results of laboratory efficacy studies for each species claimed to be controlled with a toxic bait or ground spray. The purpose of these studies is to assess the laboratory efficacy of the formulations that will be tested in the field.

(1) Test standards. Use the wild target species. Use at least 10 males and 10 females for each formulation. Use at least 10 males and 10 females for controls. Greater than 10% mortality in the controls negates the test. Provide a free choice between the rat and mouse challenge diet and the toxic bait or sprayed vegetation. The exposure period for single-

dose toxicants is 3 days. For multiple-dose toxicants, the exposure period is 15 days.

(2) Suggested performance standard. Under laboratory conditions, a mortality of 100% is desirable while 90% is suggested.

(3) Test information. Indicate the species and origin of test animals. Describe acclimation of animals and laboratory conditions. Provide daily food consumption data for each animal. Indicate the day of death for each animal. Report summary data, including the mortality and the total amount and percentage of toxic material and challenge diet consumed. Submit a chemical analysis of the test ration for the percentage of active ingredient. (See references 13 and 14 of § 96-25.)

(4) Acceptable protocols. References cited or exhibits included in § 96-30 may be helpful in developing an acceptable protocol incorporating the test standard above. For methods of chemical analysis consult references 13 and 14 of § 96-25.

(e) Field efficacy studies. Submit the results of field efficacy tests for each species claimed to be controlled with a toxic bait or ground spray in an orchard. The purpose of these studies is to assess the field efficacy of the product under actual use conditions.

(1) Test standards. (i) Number of regional studies. For each formulation, site, method of application, major region, and species claimed or implied to be controlled, submit results of five field efficacy studies.

(ii) Pre-treatment and post-treatment population censusing. Two basic methods of censusing may be used, one of which is direct and the other indirect. In direct censusing, animals are livetrapped, individually marked, released, and recaptured. (See reference 20 in § 96-25.) In indirect censusing, sections of apples are used to obtain a relative index of orchard mouse activity. (See reference 21 in § 96-25.) If this method is used, some live trapping will be necessary to substantiate the identity of the target species.

(iii) Rate, frequency and method of application. In order to provide adequate use directions on the label, efficacy testing should include trials at different rates (e.g., 1 lb. per acre or 4 oz. per placement), frequencies (e.g., 1 application per week or month), and methods of application (e.g., ground, hand, or aerial). If a claim is made that a product is effective for a particular time period, then the test design should reflect studies covering that period of time.

(iv) Effect of environmental factors and managerial practices. Studies should be conducted under the environmental factors anticipated under actual use conditions. Environmental factors such as precipation, temperature, light, humidity, and wind may have an impact on product effectiveness as well as managerial practices such as sanitation, timing of planting, and location of crops. These variables should be taken into account in the experimental test design.

(2) Test information. Indicate the formulation, and location, site, method, number, and frequency of applications for each study. Provide a map of test and control areas, including their dimensions. Report all raw and summary data used to evaluate effectiveness. Submit mortality information by species and sex on target and non-target animals in experimental areas. Indicate the method and rationale for selecting experimental areas and assigning them to test or control groups. Provide climatological data for the study period. Include all information on the statistical methods used.

(3) Acceptable protocols. For direct censusing of orchard mice, see reference 20 in § 96-25. For indirect censusing of orchard mice with apple slices, consult reference 21 in § 96-25. References 22 and 23 in § 96-25 also may be helpful in developing an acceptable protocol incorporating the test standards described in paragraph (e)(2) of this section.

§ 96-12 Rodenticides on farm and rangelands.

(a) General. This section supplies information on efficacy data and test procedures for rodenticides applied as baits on farms and rangelands. Target animals include gophers, prairie dogs, marmots, ground squirrels, and field mice. Registration guidance for feral commensal rodenticides are provided in § 96-10. Submission of efficacy data is waived for this section.

(b) Acute and subacute toxicity studies. Submit the results of toxicity studies for each species claimed to be controlled with a rodenticide applied as a bait on farm and range lands. For a single-dose bait, submit an acute oral LD50 test. For a multiple-dose bait, submit an acute oral LD50 test and a subacute (5-day) oral LD50 test. The purpose of these studies is to assess the appropriateness of dosage levels used in subsequent testing.

(1) Test standards. Use the wild target species. Use at least 10 animals for each dosage level for each sex. Cage test animals individually. Observe changes in behavior that may affect efficacy, and gross pathological changes at post mortem (e.g., hemorrhaging, edema, ulcers, discoloring). Consult a standard reference for detailed test standards. (See reference 9 of § 96-25.)

(2) Test information required. Indicate the species and origin of test animals. Describe acclimation of animals, laboratory conditions (cage specifications, temperature, humidity, light), and changes in animals' behavior that may affect efficacy. Indicate the the sex and weight of each animal. Describe gross pathological changes found at post mortem. Submit a dose-response curve for each sex with the computation of the slope and LD50 with 95% confidence limits. (See reference 5 in § 96-25.)

(c) Laboratory acceptance studies. Submit the results of laboratory acceptance studies for each species claimed to be controlled with a rodenticide applied as a bait on farms and rangelands. The purpose of these studies is to assess the appropriateness of bait carriers used on subsequent testing.

(1) Test standards. Use the wild target species. Use 6 adult males, 6 adult females, and 6 juveniles of either sex for each target species. Cage test animals individually. Provide a free choice between each candidate carrier and the habituated diet. Replace the habituated diet with the standard challenge diet when it has been established. For example, see Exhibits 2 through 20 in § 96-30. Continue recording daily food consumption data until the percentage of each food eaten stabilizes. Conduct laboratory bait acceptance studies with all candidate carriers.

(2) Test information. Indicate the species and origin of test animals. Describe acclimation of animals and laboratory conditions. Report food consumption data for each animal. Provide summary data by sex and age, including the quantity and percentage of each diet consumed.

(3) Acceptable protocols. References cited in § 96-30 may be helpful in developing an acceptable protocol incorporating the test standards described in paragraph (c)(2) of this section.

(d) Laboratory efficacy studies. Submit the results of a laboratory efficacy test for each species claimed to be controlled. The purpose of these studies is to assess the laboratory efficacy of the formulation.

(1) Test standards. Use the wild target species. Use at least 10 males and 10 females for each test. Use at least 10 males and 10 females for controls. Greater then 10% mortality in the controls negates the test. Provide a free choice between the habituated ration or the standard challenge diet and the toxic bait. The exposure period for a single-dose toxicant is 3 days. For a multiple-dose toxicant, the exposure period is 15 days. Test all formulations that may be used in the field.

(2) Suggested performance standard. In an acceptable laboratory efficacy test, the product should kill at least 90% of the test animals.

(3) Test information. Indicate the species and origin of test animals. Describe acclimation of animals and laboratory conditions. Provide daily food consumption data for each test animal. Indicate the day of death of each animal. Provide summary data by sex and species. Submit a chemical analysis of the bait for the percentage of active ingredient. (See references 13 and 14 of § 96-25.)

(4) Acceptable protocols. For methods of chemical analysis, consult references 13 and 14 in § 96-25. References cited in § 96-30 may be helpful in developing an acceptable protocol incorporating the test standards described in paragraph (d)(2) of this section.

(e) Field efficacy studies. Submit the results of field efficacy tests for each species claimed to be controlled with a rodenticide applied as a bait on farms and rangelands. The purpose of these studies is to assess the field efficacy of the product under actual use conditions.

(1) Test standards. (i) Number of regional studies. Submit 5 efficacy studies for each formulation, site, method of application, major region, and species claimed or implied to be controlled on the label.

(ii) Pre-treatment and post-treatment population censusing. Two basic methods of censusing may be used, one of which is direct and the other indirect. In direct censusing, animals may be live-trapped, individually marked, released, and recaptured. (See reference 20 in § 96-25.) Also, visual counts of rodents may be made at periodic intervals (e.g., early morning) for several days in order to obtain a relative index of rodent pressure. In indirect censusing, mark mounds or burrows individually. Depending on the species, these would be either opened or closed. After one or two days, determine which burrows still show activity. For gophers, use at least 25 active mounds in treated and control areas. (See reference 24 in § 96-25.)

(iii) Control site. A control site is needed for every study.

(iv) Rate, frequency, and method of application. Efficacy testing should include trials at different rates (e.g., 1 lb per acre or 4 oz per placement), frequencies (e.g., 1 application per week or month), and methods of application (e.g., ground, hand, or aerial). If a claim is made that a product is effective for a perticular time period, then the test design should reflect studies covering that period of time.

(v) Effect of environmental factors and managerial practices. Studies should be conducted with the environmental factors anticipated under actual use conditions. Environmental factors (such as precipitation, temperature, light, humidity, and wind), as well as managerial practices (such as sanitation and timing of planting), may have an impact on product effectiveness. These variables should be taken into account in the experimental test design.

(2) Suggested performance standards. An acceptable level of efficacy should result in at least a 70% reduction of rodent activity.

(3) Test information. Report the results of all studies. Submit a sketch of the test sites indicating the the proximity to surrounding areas which may harbor the target species. Indicate the dates of treatment. Include all raw data used for computing the population levels. Indicate the kind and amount of all toxic bait applied in pounds per acre. Report the spacing between treated areas. Describe the soil type and ground cover. Indicate the percentage of animals controlled. Submit information (mortality by species and sex) on target and non-target kills in experimental areas.

(4) Acceptable protocols. For direct censusing of rodents, consult reference 20 in § 96-25. For indirect censusing of pocket gophers, refer to reference 24 in § 96-25. For indirect censusing of other rodents, refer to § 96-30.

§ 96-13 Rodent fumigants.

(a) General. This section lists efficacy data and test procedures for rodent fumigants. Target animals include the Norway rat, roof rat, Polynesian rat, house mouse, pocket gopher, and ground squirrel. Submission of efficacy data is waived for this section.

(b) Acute toxicity studies. Submit the results of an acute inhalation LC50 toxicity test for each target animal claimed to be controlled by a rodent fumigant. The purpose of these studies is to assess the appropriateness of dosage levels to be used in subsequent testing.

(1) Test standards. Use the wild target species. Use at least 6 males and 6 females for each dosage level for pocket gophers and ground squirrels. Use at least 10 males and 10 females for each rat and mouse species. Test animals individually in a gas-tight chamber. For acceptable equipment and procedures in determining toxicity of fumigants, consult Section 4.3 of reference 16 of § 96-25. Observe changes in behavior that may affect efficacy. Observe gross pathological changes found at post mortem (e.g., hemorrhaging, edema, ulcers, discoloring). Consult a standard reference on the subject for more detailed test standards. (See reference 9 in § 96-25.)

(2) Test information. Indicate species and origin of test animals. Describe acclimation of animals, laboratory conditions (e.g., temperature, humidity, light, cage size), changes in behavior during the fumigation test that may affect efficacy, and gross pathological changes found at post mortem. Separate the mortality data by sex for each dosage level. Submit a dose response curve for each sex with the computation of the slope and LC50 with 95% confidence limits. (Check reference 5 in § 96-25.)

(c) Laboratory efficacy studies. Submit the results of a laboratory efficacy test for each species claimed or implied to be controlled with a rodent fumigant. The purpose of these studies is to assess the laboratory efficacy of formulations.

(1) Test standards. Use the wild target species. Use at least 6 adult males and 6 adult females for each dosage level for pocket gophers and ground squirrels. Use at least 10 adult males and 10 adult females for each rat and mouse species. Test the product for one hour. Test animals individually in an artificial burrow. (See reference 18 in § 96-25.) Do not fast animals prior to efficacy tests.

(2) Suggested performance standard. Under laboratory conditions, a mortality of 100% is desirable; 90% is suggested.

(3) Test information. Indicate species and origin of test animals. Describe acclimation of animals and tunnel conditions (e.g., temperature, humidity). Provide a sketch with dimensions of the tunnel and exhaust systems. Describe the type of soil surrounding tunnel systems. Record the amount of time required to kill each individual animal. Submit all raw and summary data including mortality according to sex of test animals. Submit a chemical analysis of the fumigant formulation for the percentage of active ingredients. Consult references 13 and 14 in § 96-25.

(4) Acceptable protocols. For conducting a laboratory efficacy study in an artificial burrow, see reference 18 in § 96-25. Others cited in § 96-30 may be helpful in developing an acceptable protocol incorporating the test standards described in paragraph (c)(2) of this section. For methods of chemical analysis, consult references 13 and 14 in § 96-25.

(d) Field efficacy studies. Submit field efficacy tests for each species claimed to be controlled with a rodent fumigant. The purpose of these studies is to assess the field efficacy of the product under actual use situations.

(1) Test standards. (i) Number of regional studies. Submit 5 field efficacy studies for each formulation, site, method of application, major region, and species claimed or implied to be controlled on the label.

(ii) Pre-treatment and post-treatment population censusing. Indices of animal density can be made by live trapping, food consumption, non-toxic tracking dust, opening or closing burrows, and smoked milk cartons. If live trapping is used, animals should be marked individually. If live trapping is not one of census methods, some live trapping prior to pre-treatment censusing may be necessary to confirm the presence of the pest species. If food consumption is used, there must be at least one placement inaccessible to animals, but open to the air, to determine moisture uptake. Otherwise, all baits should be dried to a constant weight prior to placement and after removal. If nontoxic tracking dust or smoked milk cartons are used, some preliminary work in the laboratory will be needed to determine how long the dust or cartons have to be in place before the rodents begin leaving tracks. [Suggested rating scale: 0 = no activity; 1 = little activity (1-3 tracks); 2 = moderate activity (4-6 tracks); and 3 = heavy activity (7 or more tracks).] For each study, pretreatment censusing should indicate the presence of at least 20 animals of the target species (for treatment of buildings) of at least 20 active burrows (for treatment of burrows) in both the test and control areas.

(iii) Site selection. Select sites that are isolated as much as possible from areas that may serve as reservoirs of reinfestation. Treat all buildings and burrows at test sites.

(iv) Rate, frequency, and method of application. All fumigants should be tested at the rate, frequency, and method of application anticipated in the use directions.

(v) Effect of environmental factors. Environmental factors such as temperature and humidity may have an impact on product effectiveness. These variables should be taken into account in the experimental test design.

(2) Suggested performance standard. Acceptable studies are those which show 70% or greater reduction in the target population based on the censusing techniques outlined in this section.

(3) Test information. Report the results of all studies. Submit a sketch of the test and control sites, indicating the location and approximate dimensions of all buildings and burrows. Also, indicate where the rodent fumigants were applied. Include all raw data used for computing population levels. Report all data on amount of fumigant applied. Record the weight, species, and sex of all target and nontarget animals found dead. Perform a post mortem on these animals to determine cause of death. Indicate the method used for marking animals.

(4) Acceptable protocols. No specific complete test protocols exist for field evaluation of rodent fumigants. However, protocols cited in § 96-30 may be helpful in developing an acceptable protocol incorporating the test standards described in paragraph (d)((2) of this section.

§ 96-14 Rodent repellents on tree seeds.

(a) General. This section indicates directions for obtaining efficacy data and test procedures for rodent repellents applied to tree seeds. Target species include deer mice. Submission of efficacy data is waived for this section.

(b) Acute toxicity studies. Submit the results of toxicity studies for each species claimed to be controlled with a repellent applied to tree seeds. These tests shall include an acute oral LD50 test. The purpose of these studies is to assess the appropriateness of dosage levels used in subsequent testing.

(1) Test standards. Use the wild target species. Use at least 10 adult males and 10 adult females for each dosage level. Cage test animals individually. Observe any changes in behavior that may affect efficacy. Observe gross pathological changes found post mortem (e.g., hemorrhaging, edema, ulcers, discoloring). For detailed information on test standards, consult a standard reference on the subject. (See reference 9 in § 96-25.)

(2) Test information. Indicate the species and origin of test animals, laboratory conditions (e.g., temperature, humidity, light, cage size), and gross pathological changes found post mortem. Separate the mortality data by sex for each dosage level. Submit a dose response curve for each sex with computation of the slope and LD50 with 95% confidence limits.

(c) Laboratory efficacy studies. Submit the results of a laboratory efficacy test for each species claimed to be controlled with a repellent applied to tree seeds. The purpose of these studies is to assess the laboratory efficacy of the formulation.

(1) Test standards. (i) Use the wild target species. Use at least 10 adult males and 10 adult females for controls. Greater than 10% mortality in the controls negates the test. Provide a free choice between the maintenance ration or the standard challenge diet and the repellent-treated seeds. Use a standard challenge diet when one has been established. For example, see Exhibits 2 through 20 in § 96-30.

(ii) The treated seeds should be of the species for which protection is claimed or implied on the label. Fifty treated seeds should be offered daily to the mice for 6 days. A seed is considered damaged if the seed coat has been cracked whether or not the endosperm has been eaten. The controls receive 50 untreated seeds daily.

(iii) Repellency is calculated according to the following formula:

$$\%\ \text{Repellency} = \left[\ 1 - \left(\frac{\text{No. seeds damaged by test animals}}{\text{No. seeds damaged by control animals}} \right) \right] \times 100$$

(iv) The consumption of the maintenance or standard diet should be recorded daily. The amount of maintenance or standard diet must exceed the daily requirement of the species tested. Do not fast animals prior to efficacy tests.

(2) Suggested performance standards. Under laboratory conditions, a 50% or greater repellency is suggested.

(3) Test information. Indicate the species and origin of test animals. Describe acclimation of animals and laboratory conditions. Report daily seed damage by each animal. Provide daily maintenance or standard challenge diet consumption data for each animal. Indicate the day of death of each animal. Provide summary data by sex and species. Submit a chemical analysis of the repellent-treated seed indicating the percentage of active ingredient. (See references 13 and 14 in § 96-25).

(4) Acceptable protocols. For conducting a laboratory efficacy study of tree seed repellents, see reference 25 in § 96-25). Other references cited in § 96-30 may be helpful in developing acceptable protocols incorporating the test standards described in paragraph (d)(2) of this section.

(e) Field efficacy studies. Submit the results of field efficacy tests for each species of rodents claimed to be repelled by repellent-treated tree seed. The purpose of these studies is to assess the field efficacy of the product under actual use situations.

(1) Test standards. (i) Number of regional studies. Submit 5 efficacy studies for each formulation, site, method of application, major region, and species claimed or implied to be controlled on the label.

(ii) Pre-treatment and post-treatment censusing in study areas. Use live traps at selected points of heavy deer mouse activity in study plots for 3 to 6 nights. All deer mice should be marked and released at site of capture. At least 12 different deer mice per 300 trap nights should be captured for acceptable test and control plots. There should be a control plot for every test plot. Test and control plots should be comparable in mouse populations, acreage, and topography characteristics. Control plots should be stocked with untreated seed at the same rate as the test site. Plot sizes should be a minimum of 10 acres and a maximum of 100 acres.

(iii) Damage evaluation. To rate repellent effectiveness, each plot should be sampled by at least 64 milacre subplots spaced uniformly. All newly germinated seedlings in the milacre should be tallied and identified. Germination assessments should be conducted in the spring and again in the summer.

(iv) Phytotoxicity characteristics. To determine any phytotoxic characteristics of the tree seed repellent, 4 replicate lots of treated and untreated seeds are to be tested in a seed germinator and monitored for 4 weeks. For additional information on phytotoxicity, consult Subdivision J.

(2) Suggested performance standard. The level of efficacy that should be attained for an acceptable test has not been established. However, seedling counts in the treated plots should average at least 50% higher than the control plots. Seedling counts should average at least 1.25 seedlings per milacre sample in the test plots.

(3) Test information. Indicate the species of test animals and tree seed in treated and control areas. Give the source of tree seeds. Report number of animals captured and total population estimate for each plot. Indicate the approximate age of animals. Describe plot conditions (e.g., size, spatial arrangement, directional orientation of plots, and soil moisture). Provide a map of the test area including directional orientation and treatment placements. Report relevant behavior of all animals (including nontarget species) interacting with the repellent. Describe the procedures of the test, application method, treatment levels, frequencies, and locations. Submit weather data (e.g., temperature, humidity, wind speed and direction, and precipitation). Report the percent repellency for each pair of test and control plots. Describe and report all statistical analyses. Report the results of phytotoxicity tests.

(4) Acceptable protocols. Reference 26 in § 96-25 and others cited in § 96-30 may be helpful in developing an acceptable protocol incorporating the test standards described in paragraph (e)(2) of this section.

§ 96-15 Rodent repellents on cables.

(a) General. This section indicates directions for obtaining efficacy data and test procedures for rodent repellents applied to underground cables. Target species include the plains pocket gopher, northern pocket gopher, and valley pocket gopher. Submission of efficacy data is waived for this section.

(b) Preliminary acute and subacute toxicity studies. Submit the results of toxicity studies for each species claimed to be controlled with a rodent repellent applied to underground cables. These studies should include an acute oral LD50 test and a subacute (5-day) oral LD50 test for the Norway rat. The purpose of these studies is to determine the mode of action of the repellent and the possibility of hazard to the target species.

(1) Test standards. Use the Norway rat. Laboratory strains of this species are acceptable. Use at least 10 adult males and 10 adult females for each dosage level. Cage test animals individually. Observe any test changes in behavior that may affect efficacy. Observe gross pathological changes found at post mortem (e.g., hemorrgaging, edema, ulcers, discoloring). For detailed information on test standards, refer to §§ 81-1 and 82-1 of Subdivision F of these document.

(2) Test information. Indicate the species, strain, and origin of test animals. Describe acclimation of animals, laboratory conditions (e.g., temperature, humidity, light, cage, size), and gross pathological changes found at post mortem. Separate the mortality data by sex for each dosage level. with 95% confidence limits. (See reference 5 of § 96-25.) For detailed information, refer to §§ 81-1 and 82-1 of Subdivision F of this document.

(3) Acceptable protocols. For conducting toxicity tests, refer to §§ 81-1 and 82-1 of Subdivision F. For presenting a dose-response curve, consult reference 5 of § 96-25.

(c) Advanced acute and subacute toxicity studies. If either test listed in paragraph (b) of this section yields an LD50 of 1000 mg/kg or less, conduct tests using the target genus. The purpose of these studies is to assess the product toxicity and mode of repellency to the target species.

(1) Test standards. Use the wild target species. Use at least 10 adult males and 10 adult females for each dosage level. Cage test animals individually. Observe any changes in behavior that may affect efficacy. Observe gross pathological changes found at post mortem (e.g., hemorrhaging, edema, ulcers, discoloring). For detailed information on test standards, consult a standard reference on the subject. (See reference 9 in § 96-25.)

(2) Test information. Indicate the species and origin of test animals. Describe acclimation of animals, laboratory conditions (e.g., temperatures, humidity, light, cage size), and gross pathological changes found at post mortem. Separate the mortality data by sex for each dosage level. Submit a dose response curve for each sex with computation of the slope and LD50 with 95% confidence limits. (Consult reference 5 in § 96-25.)

(d) Small plot efficacy studies. Submit the results of small plot efficacy studies for each species claimed to be controlled. The purpose of these studies is to assess the efficacy of formulations under small plot conditions.

(1) Test standards. Use live traps in each study area to determine the target species. Only use sites adjacent to acctive burrows. ("Active" burrows are ones that are plugged by gophers after once being opened by the investigator). Adjavent to an active burrow, install two 5-ft sections of underground cable of the type to be protected. Place cable sections in separate trenches a minimum of 3 ft apart so that treatment of one cable does not affect an untreated cable. Use 10 replications per treatment.

Keep records on all methods of application and the dosage rates. Do not reuse trenches. Test cable sections under both cool (less than 5°C) and warm (more than 20°C) weather. Test cable sections where soil is saturated with water and where soil is well drained, because efficacy of certain products is susceptible to leaching. After treatment, continue to monitor cables at least twice a week for 12 weeks. Check both treated and untreated cables. Also, according to a predetermined method acceptable to the Agency, rate the damage to the cables using teeth marks as an index.

(2) Suggested performance standard. Although no level of efficacy for an acceptable test has been established, 70% fewer teeth marks and opened circuits on treated cables, compared to untreated cables, is suggested.

(3) Test information. Indicate the target species for each study area. Include a map of all study areas, denoting location of active burrows and treated and untreated cables. Indicate the distance between all cables. Report the method and frequency of application, formulation, dosage rates, type of cable (number of circuits, type of coating, and diameter), the temperature, precipitation, and soil moisture. For each check of cables, include the raw and summary data with respect to cable damage. Describe all statistical methods used to evaluate efficacy.

(4) Acceptable protocols. References cited in § 96-30 may be helpful in developing an acceptable protocol incorporating the test standards described in paragraph (d)(2) of this section.

(e) Field efficacy studies. Submit the results of field efficacy studies for each species claimed to be controlled. The purpose of these studies is to assess the efficacy of the product under actual use conditions.

(1) Test standards. Use live traps at selected points of heavy gopher activity along the length of cable to determine the target species.

(i) Number of regional studies. For each formulation, dosage rate, method of application, and species claimed or implied to be controlled on the label, submit data from at least 50 miles of treated and 50 miles of untreated cables that are in actual use.

(ii) Pre-treatment and post-treatment population censusing. At least 6 study areas along both treated and untreated cable should be established. These areas should include 3 areas of moderate gopher pressure and 3 areas of heavy gopher pressure. Two of the areas of moderate pressure and 2 of the areas of heavy pressure should be in well-drained soil. One of the areas of moderate pressure and one of the areas of heavy pressure should be in poorly drained soil. A quantitative definition of moderate and heavy pressure must be established, based on the number of active burrows per unit area. (An "active" burrow is one that is plugged by gophers after being opened by the investigator.) At periodic intervals (e.g., every 2 months for 12 months), evaluate the gopher pressure at these sites.

(iii) Damage assessments. Damage assessments will be made at the same time that census assessments are made. Indicate the number of circuits opened using a volt-ohm meter. Also, according to a predetermined method, rate the damage to the cables using the teeth marks as an index. Finally, maintain data on the number of "blowouts" that occur along treated and untreated cable.

(iv) Records required. Keep records on the method and frequency of application, dosage rates, temperature, soil moisture, and cable type.

(2) Suggested performance standard. Though no level of efficacy for an acceptable test has been established, 70% fewer "blowouts" or number of teethmarks and opened circuits for treated cable, compared to untreated cable, is suggested.

(3) Test information. Indicate the target species at selected points along the cable. Include a map with dimensions of all study areas along treated and untreated cable. Submit a detailed map of the study areas. Indicate the method and frequency of application, formulation, dosage rates, temperature, precipitation, and soil moisture. Provide all raw and summary data upon which the censusing and damage assessments were made. Describe all statistical methods used to evaluate efficacy.

(4) Acceptable protocols. References cited in § 96-30 may be helpful in developing an acceptable protocol incorporating the test standards listed above.

§ 96-16 Rodent reproductive inhibitors.

(a) General. This section provides efficacy data and test procedures for rodent reproductive inhibitors applied as dry baits. Target animals include the Norway rat, roof rat, Polynesian rat, house mouse, and certain species of *Microtus* and *Peromyscus*. Submission of efficacy data is waived for this section.

(b) Acute and subacute toxicity studies. Submit type results of an acute oral LD_{50} test and subacute (5-day) oral LD_{50} test for each target animal claimed to be controlled with a rodent dry bait reproductive inhibitor. The purpose of these studies is to assess the appropriateness of dosage levels used in subsequent testing.

(1) Test standards. Use the target animals. Laboratory strains of target species are acceptable. Cage the test animals individually. Test both juvenile animals and adult animals (sexually mature). For each sex and age group, use at least 10 animals for each dosage level. Observe changes in behavior that may affect efficacy and gross pathological changes found at post mortem (e.g., testicular or ovarian atrophy, hemorrhaging, edema, ulcers). For detailed test standards, consult a standard reference on the subject. (See reference 9 in § 96-25.)

(2) *Test information.* Indicate the species, strain, and origin of test animals. Describe acclimation of animals, laboratory conditions (e.g., temperature, humidity, light, and cage size), changes in behavior during the test that may affect efficacy, and gross pathological changes found at post mortem, including information on reproductive inhibition. Separate the mortality data by age and sex for each dosage level. For each sex, indicate the number dying at each dosage level. Submit a dose response curve with the computation of the slope and LD_{50} with 95% confidence limits.

(c) *Laboratory acceptance studies.* Submit the results of laboratory acceptance studies for each formulation and species claimed to be controlled with a dry bait rodent reproductive inhibitor. The purpose of these studies is to assess the laboratory acceptance of the reproductive inhibitor in the presence of a standard challenge diet and to provide subjects for subsequent breeding studies.

(1) *Test standards.* Use the wild target animal. Use at least 20 males and 20 females per age group (juvenile and adult) for each test. Use at least 10 males and 10 females per age group as controls. *If the reproductive inhibitor is sex specific, use only the appropriate sex.* A standard challenge diet is provided whenever the animals are exposed to dry bait reproductive inhibitor. (See Exhibits 2-20 in § 96-30.) Cage animals individually. Test both juvenile animals and adult animals (sexually mature). Observe changes in behavior that may affect efficacy. Observe gross pathological changes found at post mortem, including information on reproductive inhibition. The exposure period should not exceed 15 days.

(2) *Test information.* Indicate the species and origin of test animals. Describe laboratory conditions. Submit the raw data on daily consumption of reproductive inhibitor and challenge diets. Indicate the weights of the animals at the beginning and end of the test, and the day of death for each animal. Provide summmary data showing the mortality (if any) and the total amount and percentage of reproductive inhibitors and challenge bait consumed. Submit a chemical analysis of the test ration for the percentage of active ingredient. (See references 13 and 14 in § 96-25.)

(3) *Acceptable protocols.* For acceptable methods of chemically analyzing products, consult references 13 and 14 in § 96-25. References cited in § 96-30 may be helpful in developing an acceptable protocol incorporating the test standards described in paragraph (c)(2) of this section.

(d) *Laboratory breeding studies.* Submit the results of laboratory breeding studies for each formulation and species claimed to be controlled with a dry bait rodent reproductive inhibitor. The purpose of these studies is to assess the reproductive success of test and control rodents.

(1) Test standards. Use the rodents tested in the laboratory feeding tests above. Use wild target animals for controls. Use at least 10 males and 10 females for each breeding test. Place each breeding pair in a separate cage with a nest box. If the reproductive inhibitor is sex-specific, treat only the appropriate sex. Breed at least 10 males and 10 females that had been treated with a reproductive inhibitor as juveniles. Breed at least 10 males and 10 females that had been treated with a reproductive inhibitor as adults. Breed at least 10 males that had been treated with a reproductive inhibitor as juveniles and 10 females that were untreated. Breed at least 10 males that had been treated with a reproductive inhibitor as adults and 10 females that were untreated. Breed at least 10 females that had been treated with a reproductive inhibitor as adults and 10 males that were untreated. Breed at least 10 untreated males and 10 untreated females to serve as controls. Observe changes in behavior that may affect efficacy. Observe gross pathological changes found at post mortem (e.g., testicular or ovarian atrophy, hemorrhaging, edema, ulcers). Continue breeding test for 90 days or until 3 separate litters have been born per breeding pair. To insure that reproductive inhibition is due to the treatment and not to sexual immaturity, juvenile subjects must be raised to sexual maturity before use in breeding tests. Sacrifice all progeny 7 days after parturition.

(2) Test information. Report the number of litters and progeny (if and when produced) from all the mating combinations specified above. Record individual weight, number, and sex of all progeny per litter at sacrifice date. Report morphological abnormalities of progeny at sacrifice date. Describe breeding cages, nest boxes, and laboratory conditions. Report the elapsed time in days from establishment of breeding pairs to the birth of the first, second, and third litters. Report weekly weights of all test and control animals from establishment of breeding pairs to end of test. Summarize the amount of reproductive inhibitor consumed according to: number of litters and progeny produced by age group; mortality (if any); and the number of litters and progeny produced, treated males, treated females, and both males and females treated. Also summarize the number of litters and progeny produced by controls. Submit a copy of all raw data.

(3) Suggested performance standards. Although an acceptable level of efficacy has not been established, a 90% reduction in reproductive success (total progeny produced), when compared to controls, is suggested.

(e) Field efficacy studies. Submit the results of field efficacy tests for each species claimed to be controlled with a reproductive inhibitor. The purpose of these studies is to assess the field efficacy of the product under actual use situations.

(1) Test standards. (i) Indoor and outdoor studies. If an indoor use is claimed, only the indoor studies are necessary. For outdoor studies, the following types of sites are acceptable: sheds (covered, non-enclosed structures), small dumps that are not being continually reinfested, and small isolated stockyards (all placements in tamper-proof bait boxes or in rodent burrows).

(ii) Number of regional studies. Norway rat: five indoor studies (one in each of the five major regions of the country) and three outdoor studies (each in a different region). Roof rat: two indoor studies (in different regions) and two outdoor studies (in different regions). House mouse: five indoor studies (one in each region) and three outdoor studies (in different regions). Microtus sp.: five outdoor studies (one in each region). Peromyscus sp.: five outdoor studies (one in each region).

(iii) Pre-treatment and post-treatment population censusing. For reproductive inhibitors, at least two methods of pre-treatment and post-treatment censusing are needed, one of which must be the mark and release method. (See § 96-30, Field Census Techniques). Some methods that will be acceptable for the second census method are food consumption, non-toxic tracking dust, smoked milk cartons, mapping of rodent signs, tracking, and electronic remote censusing. All live-trapped animals should be marked individually. If food consumption is used, there should be at least one placement inaccessible to animals, but open to the air, to determine moisture intake. Otherwise, all baits should be dried to a constant weight prior to placement and after removal. If non-toxic tracking dust or smoked milk cartons are used, some preliminary work in the laboratory will be needed to determine how long the dust or cartons have to be in place before the rodents begin leaving tracks. A suggested rating system is: 0 = no activity; 1 = little activity (1-3 tracks); 2 = moderate activity (4-6 tracks); and 3 = heavy activity (7 or more tracks). The test and control sites should be censused immediately before treatment and on a monthly basis thereafter for a year.

(iv) Site selection. Select sites that are isolated as much as possible from areas that may serve as reservoirs of reinfestation. Treat all buildings at the test sites.

(v) Treated and untreated areas. At least one untreated control site should be censused for each study. The control sites should be treated with a placebo form of the bait used at the treated sites at the same application rates. All toxic rodenticide baits should be permanently removed from the test and control sites 30 days before pre-treatment censusing.

(2) Suggested performance standards. Field studies should meet the following efficacy standards. Population levels are to be determined one year after application of the reproductive inhibitor to the test sites. Reproductive inhibitors that claim to both kill commensal rodents and reduce reproduction in the survivors should meet this parameter of efficacy.

(i) For test and control sites treated with a toxic rodenticide 3 months or less prior to application of a reproductive inhibitor in the test site, a maximum of 30% population increase of target species at the treated site and a minimum of 80% increase at the control site is acceptable.

(ii) For test and control sites treated with a toxic rodenticide more than 3 months prior to application of a reproductive inhibitor, a reduction of 70% or greater of the target species at the treated site (due to use of the product) and not more than 20% reduction of the target species at the control site is acceptable.

(3) Test information. Report the results of all studies. Submit a sketch of the test and control sites, indicating the location and approximate dimensions of all buildings. Also indicate where the baits and traps were placed. Induce all raw data used for computing population levels. Include all data on amount of reproductive inhibitor applied and consumed. Report weight, sex, identification marks, and species of all animals live trapped. For male rodents, record if the testicles are present in the scrotum or in the body cavity, and, for females, record if they are lactating and/or are obviously pregnant. Record weight, sex, identification marks, and species of all target and non-target animals found dead. Perform post mortems to determine cause of death.

(4) Acceptable protocols. No specific complete test protocol exists for field evaluation of reproductive inhibitors on rodents. References cited in § 96-30 may be helplful in developing an acceptable protocol incorporating the test standards described in paragraph (e)(2) of this section.

§ 96-17 Mammalian predacides.

(a) General. This section deals with efficacy data procedures for mamalian predacides applied as baits and fumigants. Target species include the coyote, red fox, gray fox, feral dog, and skunk. Submission of efficacy data is waived for this section.

(b) Acute and subacute toxicity studies. Submit the results of toxicity studies of each species claimed to be controlled with a mammalian predacide. For a single dose predacide, submit an acute oral LD50 test and a subacute (5-day) oral LD50 test. For a fumigant, submit an acute inhalation LC50 test. The purpose of these studies is to assess the appropriateness of dosage levels used in subsequent testing.

(1) Test standards. Use the wild target species. Use at least 12 sexually mature animals (6 males and 6 females). Cage test animals individually. Test animals must be in large enough cages to allow freedom of movement. This requirement may be computed by using the following equation: [length of target animal (in inches) + 6 inches]: 144 inches^2/ft^2 = required floor space (ft^2). (See reference 27 in § 96-25.) Use only animals that are acclimated to test condition. Stabilize diet and general condition of animals for at least 28 days prior to testing. Observe changes in behavior (e.g., vocal, chemicals or postural communication) that may affect efficacy. Examine all affected animals for gross pathological and histological changes. For detailed test standards, consult reference 9 in § 96-25.

(2) Test information. Indicate the species and origin of test animals. Describe acclimation of animals, laboratory conditions (e.g., temperature, humidity, light, cage size), changes in behavior that may affect efficacy, and gross pathological and histological changes of those animals killed by the toxicant. Separate mortality data by sex for each dosage level. Submit a dose response curve for each sex with the computation of the slope and LD50 with 95% confidence limits. (See reference 5 in § 96-25.)

(c) Laboratory efficacy studies. Submit the results of laboratory efficacy tests for each species claimed to be controlled with the predacide. The purpose of these studies is to assess the laboratory efficacy of the toxic formulation.

(1) Test standards. Use the wild target species. Use a minimum of 12 adult target animals (6 males and 6 females) and, if possible, 6 juveniles. Provide the animals with a free choice between the habituated ration and the toxic bait. References cited in § 96-30 may be helpful in developing an acceptable protocol incorporating the test standards described in this paragraph.

(2) Suggested performance standard. To date, the levels of mortality required of each predacide test have not been established. Under laboratory conditions, a mortality of 100% is preferred and a mortality of 90% is suggested.

(3) Test information. Indicate the species and origin of test animals. Describe acclimation of animals and the laboratory conditions (e.g., temperature, humidity, light, cage size). Submit raw data for consumption of toxicant bait and habituated ration. Report the following data for each affected animal: the latency period, poisoning symptoms, cause of death, and gross pathology. Submit a chemical analysis of the test ration for the percentage of active ingredient. (Consult references 13 and 14 of § 96-25 for existing methods of chemical analysis.)

(4) Acceptable protocols. References cited in § 96-30 may be helpful in developing an acceptable protocol incorporating the test standards described in paragraph (c)(2) of this section. For existing methods of chemical analysis, see references 13 and 14 of § 96-25.

(d) Field efficacy study. Submit field efficacy tests for each species claimed to be controlled with the predacide. The purpose of these studies is to assess the field efficacy of the product under actual use situations.

(1) Test standards. Precise field test standards are not well defined because of the inherent variables (climate, animal movement, behavior modification, availability of food, habitat types, and reproductive rates). Small plot testing should precede general field testing.

(i) Number or regional studies. For each region where control of the animals is anticipated, submit five field efficacy studies for each pattern of use.

(ii) Pre-treatment and post-treatment population censusing. Indices of animal density in the area can be determined by the amount of food consumed or by the animal tracks occurring at daily monitored sites. Additional information should include data concerning livestock or wildlife depredation or disease incidence such as rabies and plague.

(iii) Site selection. Study sites should be representative of those areas where eventual use of the predacide is intended and should be relatively free from animal immigration and emigration. At least one untreated control site should be censused for each study.

(2) Suggested performance standard. Acceptable efficacy studies are those which show sufficient predator reduction (due to use of the product) as to bring about a cessation or satisfactory reduction in livestock or wildlife depredation or an interruption of disease incidence such as rabies and plague.

(3) Test information. Submit results of all studies. Indicate the size of area involved and test site legal locations using U.S. Geological Survey map coordinates. Identify target species and their relative population densities and locations. Report the number and amount of toxic baits and/or devices placed per acre, square mile, or township. Indicate the number and distribution of target and non-target species found dead or seen affected. Perform post mortems to determine cause of death. Submit all raw data used for computing population levels, livestock depredation, and disease incidence.

(4) Acceptable protocols. No specific complete test protocol exists for field evaluation of toxic chemicals for predator management. References cited in § 96-30 may be helpful in developing an acceptable protocol incorporating the test standards described in paragraph (d)(2) of this section

§ 96-18 Domestic dog and cat repellents.

(a) General. This section deals with efficacy data and test procedures for domestic dog and cat repellents. Target species are the domestic dog and cat. Submission of efficacy data is waived for this section.

(b) Preliminary acute and subacute toxicity studies. Submit the results of toxicity studies for each species claimed to be controlled with a repellent. These studies should include an acute oral LD50 test and a subacute (5-day) oral LD50 test for the Norway rat. The purpose of these studies is to assess the possibility of hazard to the target species.

(1) Test standards. Use the Norway rat. Laboratory strains of this species are acceptable. Use at least 10 adult males and 10 adult females for each dosage level. Cage test animals individually. Observe any changes in behavior that may affect efficacy and note any gross pathological changes found at post mortem (e.g., hemorrhaging, edema, ulcers, discoloring). For detailed information on test standards, refer to §§ 81-1 and 82-1 in Subdivision F of this document.

(2) Test information. Indicate the strain and origin of test animals. Describe acclimation of animals, laboratory conditions (temperature, humidity, light, cage size), and gross pathological changes found at post mortem. Separate the mortality data by sex for each dosage level. Submit a dose response curve for each sex with computation of the slope and LD50 with 95% confidence limits. For detailed information on test information, refer to §§ 81-1 and 82-1 in Subdivision F of this document.

(3) Acceptable protocols. For conducting toxicity tests, refer to §§ 81-1 and 82-1 in Subdivision F of this document. For presenting a dose-response curve, consult reference 5 in § 96-25.

(c) Advanced acute and subacute toxicity studies. If either test conducted as required in paragraph (b) of this section yields and LD50 of 1000 mg/kg or less, conduct the tests using the target species. The purpose of these studies is to assess the hazard to the target species.

(1) Test standards. Use the target species. For each sex, use at least six animals for each dosage level. Cage test animals individually. Place animals in large enough cages to provide freedom of movement. [See § 96-17 (b)(2).] Stabilize diet and general condition of animals for at least 28 days prior to testing. Observe any gross pathological changes found at post mortem (e.g., hemorrhaging, edema, ulcers, discoloring). For detailed test standards, consult a standard reference on the subject. (See reference 9 in § 96-25.)

(2) Test information. Indicate the species, breed, and origin of test animals. Describe acclimation of animals, laboratory conditions, (e.g., temperature, humidity, light, cage size), and gross pathological changes found at post mortem. Separate mortality data for each sex at each dosage level. Submit a dose response curve with computation of the slope and LD50 with 95% confidence limits. (See reference 5 in § 96-25).

(d) Pen efficacy studies. Submit the results of a laboratory efficacy test for each formulation and target species claimed to be controlled. The purpose of these studies is to assess the pen efficacy of the formulation.

(1) Test standards. (i) A separate test protocol should be used for each type of behavior claimed to be reduced (e.g., chewing and tearing garbage bags; feeding from garbage cans; and urinating, defecating, walking, and lying in specified areas).

(ii) Use the target species. Use at least 8 test animals. An equal ratio of males and females is recommended except when testing the claim to reduce urination on established scent posts by dogs, in which case only males should be used. Test animals that have had previous repellent experience may be used.

(iii) The tester must define repellency in quantitative terms. An example of an acceptable definition is: the apparent percent reduction of a specific behavior that is the result of the treatment but not as the ratio between observations on the untreated object versus treated objects.

(iv) Animals should be aclimated prior to testing. Extensive pretreatment data collection should be made to establish the baseline behavior against which to evaluate the treatment.

(v) The following factors should be taken into consideration in designing an experimental protocol: pens, test animals, test days, and treatments.

(vi) Only one formulation and treatment level can be evaluated during each test. Each test should be replicated.

(vii) Two breeds of the target species should be tested. When dogs are the target species, a complete test should be conducted with at least 2 breeds. At least one breed should be from one of the following categories of breeds designated by the American Kennel Club: hound, sporting dog, toy, terrier, working dog, or non-sporting dog. Mongrels may be considered a separate "breed".

(viii) Females in estrous or proestrous may be housed a minimum of 400 m (437 yd) from the test pens, or be housed indoors.

(ix) When cats are the target species, a complete test should be conducted on at least 2 breeds. The alley cat (mixed breed) can be considered one breed.

(x) The protocol should be designed so that no significant repellent contamination of the test pen remains from previous tests.

(xi) The number of days a test should be conducted will vary according to the number of animals used. However, it probably should not exceed 7 days per test.

(2) Suggested performance standard. Using the definition of repellency stated in paragraph d(2)(iii) of this section, a minimum repellency of 50% due to use of the product would be acceptable, although no mandatory standards have been set. The statistical analysis should consist of a one-tailed t-test to determine if the formulation's performance if significantly greater than a set percentage (minimum of 50%).

(3) Test information. Indicate the species, breed (if known), and source of the test animals. Indicate the number, sex, and approximate age of each animal. Describe pen conditions (size, spatial arrangement, and directional orientation of pens). Describe the protocol in detail, including but not limited to the following: procedures of test, treatment level, pen layout with dimensions, treatment locations, and statistical design. Report weather data (temperature, humidity, wind speed and direction, and precipitation). Define the nature of the product's repellent action. Report treatment level and formulation, repellency for each test and breed, and complete statistical analysis.

(4) Acceptable protocols. Refer to Exhibit 21 in § 96-30 for a protocol to evaluate the efficacy of aerosol dog repellents that claim to reduce damage to garbage bags. With minor modification, this method could be used to evaluate granular products to be used around garbage bags and for products claiming to reduce feeding in garbage cans. Also, see reference 28 in § 96-25.

(e) Field efficacy studies. Submit the results of field efficacy tests for each formulation, behavior listed in paragraph (d)(2)(ii) of this section, and for each target species claimed to be controlled (repelled). In addition, for products to be applied on or near plants, consult Subdivision J for further data guidance on phytotoxicity. The purpose of these studies is to assess the field efficacy of the product under actual use conditions.

(1) Test standards. Use the target species. Repellency should be quantitatively defined. Repellency can be defined as: the apparent percent reduction of a specific behavior that is the result of treatment but not as the ratio between observations on the untreated versus treated objects. Extensive pre-treatment data collection should be made to establish baseline behavior against which to evaluate post-treatment data. The test should be designed for effective statistical analysis. Tests should be conducted in 2 different areas (e.g., rural, urban, and suburban). Separate tests should be performed during cool (mean tempature less than 5°C) and warm weather (mean temperature more than 20°C. Census the population level of the target species before and after treatment. See reference 29 in § 96-25 for dog population census methods. Studies should be conducted under actual use conditions.

(2) Suggested performance standard. While no repellency standard has been established for field studies, at least 50% repellency due to use of the product would be needed.

(3) Test information. For each study, indicate the formulation, specific location, kind of site (e.g., garbage bags, garbage cans, lawns), behavior discouraged, experimental design, test procedures, weather conditions during study, areas (rural, urban, suburban), and other relevant information. Report relevant behavior observed of all animals seen interacting with the repellent. Provide a map of the test area, including directional orientation, treatment placements, and dimensions. Provide all raw data and summary data used to evaluate effectiveness. Describe method of calculating repellency. Report percent repellency for each observation period and each test. Provide complete statistical analysis.

(4) Acceptable protocols. No protocols for field evaluation of dog and cat repellents are presently available. Prior consultation with the Agency on proposed protocols is strongly recommended. For censusing dog populations, consult reference 29 in § 96-25.

§ 96-19 Browsing animal repellents.

(a) General. This section gives information on efficacy data and test procedures for browsing animal repellents used on conifers, nursery trees, and orchard trees. Target animals include deer, elk, and lagomorphs. Submission of efficacy data is waived for this section.

(b) Preliminary acute and subacute toxicity studies. Submit the results of toxicity studies for each species claimed to be controlled with a browsing repellent. These studies include an acute oral LD50 test and a subacute (5-day) oral test for the Norway rat. The purpose of these studies is to assess the possibility of hazard to the target species.

(1) Test standards. Use the Norway rat. Laboratory strains of this species are acceptable. For each sex, use at least 10 adult males and 10 females for each dosage level. Cage test animals individually. Observe any changes in behavior that may affect efficacy, and any gross pathological changes found at post mortem (e.g., hemorrhaging, edema, ulcers, discoloring). For detailed information on test standards, refer to §§ 81-1 and 82-1 in Subdivision F.

(2) Test information. Indicate the species, strain, and origin of test animals. Describe acclimation of animals, laboratory conditions (e.g., temperature, humidity, light, cage size), and any gross pathological changes found at post mortem. Separate the mortality data by sex for each dose level. Submit a dose response curve for each sex with computation of the slope and LD50 with 95% confidence limits. (See reference 5 of § 96-25.) For details on test information, refer to §§ 81-1 and 82-1 in Subdivision F of this document.

(3) Acceptable protocols. For conducting the toxicity tests, refer to §§ 81-1 and 82-1 in Subdivision F. For presenting a dose response curve, check reference 5 in § 96-25.

(c) Advanced acute and subacute toxicity studies. If either test conducted under paragraph (b) of this section yields an LD50 of 1000 mg/kg or less, conduct the following tests using the target species. The purpose of these studies is to assess the hazard to the target species.

(1) Test standards. Use the target species. Use at least 6 mature animals of each sex per dose level. Cage or pen test animals individually. Place animals in large enough cages or pens to provide freedom of movement. Stablize diet and general condition of animals for at least 2 weeks prior to testing. Observe any changes in behavior that may affect efficacy. Observe any gross pathological changes found at post mortem (e.g., hemmorrhaging, edema, ulcers, discoloring). For detailed test standards, consult a standard reference such as reference 9 in § 96-25.

(2) Test information. Indicate the species and origin of test animals. Describe acclimation of animals, cage or pen conditions (e.g., temperature, humidity, light, cage size), and note changes in behavior during the test that may affect efficacy. Separate mortality data by each dosage level. Submit a dose response curve for each sex with the computation of the slope and LD50 with 95% confidence limits as described in reference 5 in § 96-25.

(d) Cage or pen efficacy tests. Submit the results of a cage or pen efficacy test for each species claimed to be controlled. The purpose of these tests is to assess the cage or pen efficacy of the product.

(1) Test standards. (i) Use the target species. Use at least 15 test animals, as suggested in reference 30 of § 96-25. Test animals that have had previous repellent experience may be used.

(ii) Repellency should be quantitatively defined. Repellency can be defined as the apparent percent reduction of a specific behavior that is the result of treatment.

(iii) Test animals should be acclimated to test conditions for at least 2 weeks prior to testing. Pre-treatment data collection (browse damage) should be made to establish baseline behavior against which to evaluate the treatment.

(iv) The recommended pen size is 1 acre for lagomorphs, 2 1/2 acres for deer, and 5 acres for elk. The area should be enclosed with poultry wire to reduce predation on lagomorphs. All vegetation that would ordinarily supply cover should be removed. Artificial cover for individual lagomorphs should be provided throughout the enclosure.

(v) For testing deer and elk, the enclosures should contain suitable cover for loafing, but should have little natural food.

(vi) Animals are to be fed a formulated diet as described in reference 31 of § 96-35. For testing lagomorphs, a balanced ration should be supplied.

(vii) For conifers, randomize block within animal enclosures (ten 30- by 48-foot rectangles contaning 16 rows of 10 trees planted at 3-foot intervals). Select trees in each block to reduce the row-feeding effect of penned animals. Use an equal number of treated and untreated trees.

(viii) To test any phytotoxic characteristic of the repellent, 25 trees should be simultaneously treated and evaluated throughout a growing season for foliar effects. For additional information of phytotoxicity data, consult Subdivision J of this document.

(ix) Conduct 2 repellent tests, each containing 8 treatments of 100 conifers or 30 orchard trees. Examine plots periodically until the untreated trees show 60 to 80% animal damage. Then terminate the test. Assess damage by noting whether or not test trees have been clipped, browsed, or pulled out of the ground. The percentage damage for each treatment is based on the ratio of damaged to undamaged trees for each 10-tree group per block. Conduct an analysis of variance as described in reference 30 of § 96-25.

(2) Suggested performance standard. The level of efficacy that should be attained for an acceptable test has not been established. However, a 50% reduction in damage due to use of the product is suggested.

(3) Test information. Indicate the species of test animals and trees in treated and control areas. Give the source of test animals and trees. Report the number of test animals of each sex. Indicate the approximate age of test animals and trees. Describe pen conditions (e.g., size, spatial arrangement, and directional orientation of pens). Describe the procedure of the test, treatment level, and locations. Submit weather data (e.g., temperature, humidity, wind speed and direction, and precipitation). Report the percent damage for each treatment group of 10 trees per block, and the percent repellency for all tests. Indicate the treatment level, formulation, and application method. Report the complete statistical analysis (analysis of variance). Provide the results of phytotoxicity tests.

(4) Acceptable protocols. References 30 and 31 in § 96-25 and others cited in § 96-30 may be helpful in developing an acceptable protocol incorporating the test standards described in paragraph (d)(2) of this section.

(e) Field efficacy studies. Submit the results of a field efficacy test for each species claimed. The purpose of these studies is to assess the field efficacy of the product under actual use conditions.

(1) Test standards. (i) Number of regional studies. For each formulation, method of application, major region, type of tree, and species claimed or implied to be controlled on the label, submit the results of at least 5 acceptable field effiacy studies. Two of these studies should be conducted during winter, and at least 2 should be conducted during early spring.

(ii) Repellency. Repellency should be quantitatively defined. Repellency can be defined as: the apparent percent reduction of a specific behavior that is the result of the treatment.

(iii) Site selection. For conifers, conduct the test on a reforested site where seedlings are under 2 years of age. For orchard and nursery trees, conduct the test on sites that are experiencing animal damage.

(iv) Pre-treatment and post-treatment population censusing. Extensive pre-treatment data collection should be made to establish baseline damage calculations against which to evaluate post-treatment data (i.e., the amount of tree damage before and after treatment). Lay out a series of line transects at least 1 mile (1.6 km) in length, and establish plots in treated and untreated areas as described in reference 32 in § 96-25. Also, refer to Field census techniques in § 96-30.

(v) Damage evaluation. Assess damage by noting whether or not test trees have been clipped, browsed, or pulled out of the ground. The percentage damage for each treatment is based on the ratio of damaged to undamaged trees for each transect. Use appropriate statistical analysis. Report the percent repellency for all tests. Report the results of phytotoxicity tests.

(2) Suggested performance standard. The level of efficacy that should be attained for an acceptable test has not been established. However, a 50% reduction in damage due to the use of the product is suggested.

(3) Test information. Indicate the target species and its relative abundance in the treated and untreated areas. Report the percentage of repellency attained for each formulation. Describe weather conditions during the study. Report the method of calculating repellency. Compare seedling browsing damage in treated and untreated areas. Provide a map of the test area, including directional orientation and teatment placements. Report relevant behavior of all animals seen interacting with the repellent. Provide all raw and summary data used to evaluate effectiveness, and a complete statistical analysis.

(4) Acceptable protocols. Reference 32 in § 96-25 and others cited in § 96-30 may be helpful in developing an acceptable protocol incorporating the test standards described in paragraph (e)(2) of this section.

§ 96-25 References for §§ 96-2 through -19.

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§ 96-30 Methods and protocols.

(a) Scope. This section represents an appraisal of current methodology for evaluating the efficacy of vertebrate control agents. The classes of agents scrutinized are toxicants, repellents (including aversive conditioners), reproductive inhibitors, anesthetizing chemicals, and devices. The mode of action of the control agents could not be examined on an agent-by-agent basis, especially with respect to the chemical senses. The vertebrate species include fish, amphibians, reptiles, birds and mammals. The text is arranged in phylogenetic order, with any necessary introductory explanation about the methods preceeding each target species.

The appraisal primarily evaluates, or references, methods that have been tested and published in journals and technical bulletins; some information was derived from unpublished reports and manuscripts. The fact that only limited research has been conducted to test many vertebrate control agents accounts for the paucity of citations in some areas. Also, control agents for some species, such as bears, have not been extensively studied because the damage caused by the animal to human property is limited.

Most target groups lack well documented, sound experimental protocols at this time. A great deal more research is needed in some areas before complete acceptable testing protocols can be defined.

I. Fish toxicants, repellents, and reproductive inhibitors.

A review of the needs to control fish and possible physical, biological, and chemical controls has been published by Lennon (1970). More specific reviews on fish toxicants and the information necessary for registration of a product have also been published (Lennon et al., 1970; Schnick, 1972, Marking, 1975; and Lennon and Walker, 1964).

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A. Fish toxicants.

Laboratory studies permit the establishment of baseline data on a product under controlled conditions. Possible synergistic and/or antagonistic action between the ingredients in the product can be evaluated without the variables expected in field conditions.

Guidelines for aquatic toxicity testing are published jointly by the American Public Health Association, the American Water Works Associations, and the Water Pollution Control Federation (1976). These guidelines have been carefully evaluated and revised frequently over the past 50 years. Several detailed test methods have been published in Stephan et al. (1975). Deviations from these methods should not be made unless there is considerable reason to do so. Until fairly recently, the static toxicity test was the only technique considered, but now flow-through toxicity tests are the preferred method. The latter method affords less difficulty in maintaining an adequate supply of dissolved oxygen, permitting only low metabolite accumulation for the test animals, and greater ability to maintain constant toxicant concentrations and constant water temperatures (Sprague, 1969; Sinley, 1973a). Continuous-flow bioassays require more equipment, but trouble-free equipment has been designed (Mount and Brungs, 1967; Sprague, 1969; Sinley, 1973b). A labor-saving jar rinser, a jar emptier, and an automatic liquid measuring vessel have been described for static bioassay (Hesselberg and Burress, 1967).

The referenced methods suggest using the receiving water as the diluent, but in preliminary testing of a piscicide this requirement would not be necessary. However, the researcher should consider the effect of water quality on fish health and on the toxicity of the piscicide being tested so that misleading results will not be obtained (Doudoroff, 1957; Doudoroff and Katz, 1950 and 1953; Hoar and Randall, 1971; Applegate and King, 1962; Slonim and Slonim, 1973; Murphy, 1970). Factors that should be considered in laboratory testing are listed below.

1. Because temperature will have an important effect, bioassays should be conducted at several different temperatures (Macek et al., 1969).

2. All fish for a given trial should be obtained from the same source. Fish from the same source should have been exposed to the same environmental conditions (pesticide levels, water quality, temperature, etc.) and similar handling procedures. Species or strains of fish known to be pesticide-resistant should not be used (Dziuk and Plapp, 1973; Ferguson and Bingham, 1966).

3. Although all life stages of the fish should be tested eventually (Olson and Marking, 1973; Garrison, 1968), only fish of nearly equal size should be tested in a given trial (Murphy, 1971).

4. Because mud will alter the toxicity of the compound in field trials (Ferguson et al., 1965), it may be desirable to conduct some tests in containers with varying amounts of mud (Loeb and Stackey, 1966).

5. In addition to testing the toxicity to the target fish, the effect on other vertebrates (Brooks and Price, 1961; MacPhee and Ruelle, 1969b), on representative invertebrates (Schoettger and Olive, 1961) and plants should also be determined.

6. The rate of deactivation of the piscicide should be determined (Marking and Walker, 1973; Marking and Dawson, 1972; Lee et al., 1971; and Loeb and Engstrom-Heg, 1971).

Suggested reviews of toxicity tests on specific piscicides include Berger et al., 1969; Herr et al., 1967; Haag, 1931; and MacPhee and Ruelle, 1969b.

In acute toxicity test research, results require special statistical treatment. Probit analysis is commonly used (Finney, 1947; and Litchfield and Wilcoxon, 1949), but a discussion of other statistical analyses has been written by Finney (1964). Rachlin and Perlmutter (1968) present an approach for establishing maximum permissible concentrations.

Some of the testing being required in this portion of the Product Performance Subdivision is required in Subdivisions D, E, and F, which should be reviewed for additional information. For instance, tests designed to evaluate the basic efficacy of the active ingredients in the laboratory can be used to determine the toxicity classification of the pesticide. The "References" paragraph in this section lists several excellent publications that provide information as to test methods and data requirements (Stephen et al., 1975; Standard Methods, 1976; Lennon and Walker, 1964).

Field studies.

Protocols for field evaluations have been developed by the U.S. Fish and Wildlife Service Fish Control Laboratories (Cumming, 1972). In addition, Lennon and Berger (1970) and Gilderhaus et al., (1969) have summarized field testing conducted to gain registration of a particular piscicide.

Exhibit I of this section is a "Proposed Recommended Practice for Determining Efficacy of Fish Toxicants in Static Freshwater Field Trials". It was prepared by the ASTM E-35.18 task group dealing with aquatic vertebrate pest control products. This group, composed of U.S. Dept. of Int. Fish and Wildlife Service and EPA personnel, and other interested individuals, is working through the ASTM E-35 Committee on Pesticides to develop a consensus method for evaluating aquatic vertebrate pest control agents. This draft will undergo some further revision before becoming a standard. However, it is being included in this section as a guide to registrants and Agency registration personnel because it is the only known practice that discusses the subject in detail. Equipment for adding chemicals and monitoring the effect has been described by several researchers (Anderson, 1962; Price and Haas, 1963; Ziebell, 1966; Richard, 1962).

A major problem in field applications is the effect that different water qualities have on the toxicity. Howell and Marquette (1962) suggest that a test should first be conducted on a sample of each body of water before adding the toxicant to the treatment area; other researchers have added the toxicant on the basis of laboratory studies and obtained erratic results (Huish, 1961; Mayhew, 1960; Callahan and Huish, 1968). Other problems in field applications are discussed in publications by Cushing and Olive (1956), Torblaa (1968), Kiser et al., (1963), Hughes and Lee (1973), Koeppe (1961), Bonn and Holbert (1961), and Cohen et al. (1960).

Because such a variety of organisms are involved in field applications, samples may have to be preserved for later analysis at perhaps several laboratories. Cope (1960) suggests methods for collection. Problems of homogenization of samples have been discussed by Benville (1970) and Daniels et al. (1965). Problems with extraction of pesticides are indicated by Hesselberg and Johnson (1972), Hughes et al., (1970), and Benevue and Beckman (1966).

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B. Fish repellents.

Basic toxicity of the repellent to target and nontarget species can be determined using methods described above for piscicides such as static jar and flow-through techniques (Stephan et al. 1975). There is little information published on fish repellents concerning individual and group behavior, and rate, duration, and significance of response. There is even less information available on standards and protocols for evaluating fish repellents.

Summerfelt and Lewis (1967) published a summary on fish repellents. Their paper, along with one by Hansen et al. (1972), described apparatus for laboratory testing of repellents. Four other papers describe a mixture of laboratory and field testing of repellents to migrating salmon (Brett and MacKinnon, 1954; Alderdice et al., 1954; Idler et al., 1956; Idler et al., 1961), but none of these constitute a protocol for testing repellents. The alternative is to adapt the protocols developed for piscicides (Cherry et al., 1975).

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C. Fish reproductive inhibitors.

No publications pertaining directly to fish reproductive inhibitors have been identified. Basic information on the reproductive physiology of fishes is available in 2 publications (Hoar and Randall, 1969; and Schreck, 1974). Although the research to control sex in fish discussed by Schreck was not very successful, such control would inhibit reproduction.

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EXHIBIT 1 - Proposed recommended practice for determining efficacy of fish toxicants in static freshwater field trials

1. Scope.

1.1 This recommended practice is to be used in testing the efficacy of fish toxicants in actual use conditions according to the intended use pattern. Field trials should be conducted only after laboratory tests for efficiacy and safety have been performed. Local authorities must be contacted and proper permission obtained before a field trial should be conducted.

2. Choice of trial site.

2.1 The trial site should be chosen to expedite the objectives of the test. Testing of a particular agent must be done in at least three widely separated geographical areas. Sites should be chosen to permit evaluation of biological, chemical, and physical variables on the efficacy of the fish toxicant.

2.2 Trial sites should be restricted to those in which public access can be completely controlled and in which results can be easily evaluated. Ponds which can be completely drained are preferred. All trial sites should have physical characteristics which will permit effective means of fish population sampling with electrofishing gear and nets, if the site cannot be drained.

2.3 Precautions should be taken to insure the isolation of the trial site from immigration by outside species of fish. Natural or other existing barriers should be used in preference to block nets or similar temporary barriers.

3. Site characteristics.

3.1 Field trials of this kind should not be conducted where populations of endangered species exist. Consult State Fish and Game agencies for specific information.

3.2 An accurate map of the surface outline of the lakes or ponds used should be obtained from existing sources or constructed by appropriate methods (1).

3.3 The volume of lakes shall be computed by the contour method (1). The volume of ponds may be computed by the contour method or by multiplying the surface area by the mean depth. The flow rate, if any, through the impoundment must be determined. The frequency of seasonal turnover should be documented.

3.4 The percent of bottom composed of different soil types should be estimated (2).

3.5 The immediate watershed and tributaries affecting the trial site should be identified.

4. Water characteristics.

4.1 Chemical characteristics. General chemical analyses of water should be conducted within 14 days of treatments. These analyses should include those for pH, dissolved oxygen, carbon dioxide, total alkalinity, total hardness, and any other ion or molecule suspected of influencing the activity of the toxicant under study. A sample of water taken at the time of treatment should be saved for more detailed analysis to help define the cause of any highly aberrant results in the trial. The pH should be measured near the surface, at mid-depth, and near the bottom. Dissolved oxygen should be measured at appropriate depth intervals to define the zones which have adequate oxygen for fish (5 mg/l or more), marginal oxygen for fish (less than 5 mg/l), and anaerobic zones (concentration of oxygen lower than the accuracy of the method of analysis). Oxygen concentration and pH should be monitored until the desired activity of the chemical is complete. Monitoring of pH should be continued during any longer studies on the degradation of the toxicant. Chemical analyses are to be done according to Standard methods (3) with the exceptions that pH and dissolved oxygen may be measured with electronic meters. The methods should be accurate to within 0.2 units for pH and 1.0 mg/l for oxygen.

4.2 Physical characteristics. Pretreatment measurements should include temperature, transparency (as indicated by Secchi disk), color (visual), and conductivity. Temperature should be recorded to nearest 1°C at appropriate intervals of depth to document the gradient from surface to bottom and define the bounds of a thermocline if present. Conductivity should be measured at the surface, corrected to 25°C. Water temperatures should be monitored throughout the period of the trial.

4.3 Pollution. Sources of pollution in the trial site waters must be documented and must be avoided if possible, unless they are included in the practice objectives.

5. Target fish species.

5.1 Field trials of this kind must not be conducted where populations of endangered species exist. Consult local game and fish authorities for specific information and permission.

5.2 The existing fish population must be assessed prior to treatment by appropriate methods (4) and (5) in terms of species, relative numbers, and sizes. Sampling methods generally include electrofishing, gill netting, fyke netting, and seining. The sampling before any trial should be adequate to define the most resistant target fish present. The most resistant target fish is usually one criterion by which the concentration of chemical is chosen. For purposes of assessing results of a trial, one or more of the following methods may be used:

(1) Comparison of catch per unit of effort before and after treatment by one or more types of gear in selected locations.

(2) Marking and restocking of fish captured from the existing population and comparing number recovered after treatment with total number marked.

(3) Stocking known numbers of new fish which are either marked or of a species not existing in the body of water, and comparing numbers recovered after treatment with numbers stocked. Use of some caged fish can be a valuable addition to any of these methods.

5.3 General toxicants. The fish sampling for a trial of a general toxicant should be much more intensive after treatment than before because it is difficult to capture fish if only a few remain alive.

5.4 Selective toxicants. The sampling effort will generally be more intensive in a trial where a selective kill by species or size is desired than in one where a total kill is desired. The trial of a selective toxicant should include adequate sampling to determine the percentage of the population consisting of the target fish before and after treatment.

5.5. Safe water check. Generally it is desirable to determine when the chemical has been degraded, inactivated, or diluted to nontoxic levels so the water may be restocked with fish or declared safe for other uses. Fish are caged at selected depths to determine when the entire water column is nontoxic. Small bluegills, rainbow trout, fathead minnows, or the most sensitive native fish in the area are used in these tests.

Ten or more specimens are caged at each depth. Care must be taken to assure gradual acclimation of the fish to the test conditions. Survival of 70-80% of the test specimens in each cage for 24 to 96 hours (depending on water temperature and the activity of the chemical) generally is sufficient evidence that the water is safe for fish.

6. Nontarget organisms.

6.1 Gross visual observations of acute effects on nontarget organisms should be made during all periods of observation. Generally, to satisfy requirements for registration, at least one field trial of a toxicant must include a detailed study of the effects on aquatic invertebrates.

6.2 Trials to determine effects on nontarget organisms must be conducted in the same manner as those used to determine efficacy to target organisms. In most cases, these two trials can be conducted simultaneously in similar areas on both target and nontarget animals.

6.3 The evaluation of effects of a toxicant on aquatic invertebrates will vary according to the nature of the toxicant, the anticipated effects, and the types of organisms present. Evaluation studies should be designed to define:

(1) the significant acute effect occurring within two weeks after treatment,

(2) the rate of recovery of the population in the event that there are signicant effects, and

(3) long-term effects such as disappearance of major species or group. The samples to define (1) (above) should be made within 14 days of treatment and at about 1, 7 and 14 days after treatment. Sampling to define (2) and (3) (above) should be started during the earliest time of normal population peaks after treatment.

The peaks occur in the spring and in the fall. Sampling in mid-April, mid-May, mid-June, and again in mid-September and mid-October should give valid samples of most major organisms included in northern climes. The schedule should be modified to accomodate the population cycles in southern areas. As an example, a trial conducted in late October would include pre-treatment and post-treatment sampling of 1, 7, and 14 days from the treatment date. If significant changes in number or diversity occur, the next sampling would be done in mid-April, mid-May, and mid-June of the following year. If the latter samples show sustained or recovered populations on the basis of numbers of individuals, numbers of taxa, and species diversity, further sampling would not be necessary. If necessary, sampling should be continued until 1 year after treatment, in this case to include mid-September and mid-October of that year.

6.3.1 Plankton should be sampled and analyzed using (1) and (7) as guidelines. Any modifications of these methods must be documented. The same methods must be used for all samples taken in a single study. Samples should be taken in representative areas of the body of water. In shallow or unstratified impoundments, samples should be taken subsurface and near the bottom. In stratified lakes, additional samples should be taken just above and below the thermocline. A minimum of 20 liters of water should be filtered for each sample. A diversity index (7), based on lowest possible taxon, shall be used as one of the parameters by which to compare samples from different time intervals.

These peaks occur in the spring and in the fall. Sampling in mid-April, mid-June, and again in mid-September and mid-October should give valid samples of most major organisms included in northern climes. The schedule should be modified to accommodate the populations cycles in southern areas. As an example, a trial conducted in late October would include pre-treatment and post-treatment sampling of 1, 7 and 14 days from the treatment date. If significant changes in number or diversity occur, the next sampling would be done in mid-April, mid-May, and mid-June of the following year. If the latter samples showed sustained or recovered populations on the basis of numbers of individuals, numbers of taxa, and species diversity, further sampling would not be necessary. If necessary, sampling should be continued until 1 year after treatment, in this case to include mid-September or mid-October of that year. Primary productivity and periphyton techniques should be used in a field test involving a toxicant that may be persistent, hazardous, or both, to nontarget organisms.

6.3.2 Benthic invertebrates should be sampled on the same schedule as plankton. The preferred samplers are Petersen or Ponar dredges. Sampling and analysis of samples should be done using (1) and (7) as guidelines, and the methodology should remain constant during any one study. Enough samples should be taken from different substrates and depths to permit statistical comparisons between sampling days. Pooled samples used for computation of species diversity should contain a minimum of 100 organisms. Diversity index (7), based on the lowest possible taxon, shall be used as one comparison between sampling periods.

6.4 Visual observations should be made for any effect of the toxicant on amphibians, reptiles, birds, and mammals inhabiting the immediate area of the trial site.

7. Application of toxicant.

7.1 Approximate range of concentration of the toxicant for a particular field trial will be determined from previously obtained laboratory data on the activity of the chemical in relation to water quality and species of fish present. Ideally, a series of two or three similar ponds should be treated at different concentrations to closely bracket the correct concentrations for that particular set of environmental variables and fish present. If there are unusual water qualities or species of fish present, or if there is considerable doubt as to effective concentrations, the proper concentration should be determined in field bioasssays by the methods of Burress (6) or a comparable method. Field bioassays are usually needed to determine the lowest effective and the highest safe concentration for a selective treatment. All field testing should be preceded by laboratory testing.

7.2 Compute the amount of chemical needed based on the concentration indicated by the field bioassay and the volume of water in the target area. The concentration of toxicant in the water at the test site should be reported based on active ingredient and also on total formulation.

7.3 The formulation of the toxicant used in field trials should be the identical formulation intended for registration. Data on one formulation is not usually applicable to registration of another similar formulation.

7.4 Special care should be taken to get the best possible horizontal and vertical distribution of the toxicant in the water column.

8. Equipment.

8.1 The equipment used to apply the toxicant should be well suited to the particular formulation. A complete list of equipment needed for the various phases of the trial should be prepared in advance. Include all items of personal, sampling, application, safety, and bioassay gear.

8.2 A complete documentation of equipment used in each phase of the field trial should be reported. This report should include, if possible, evidence of calibration and standardization consistent with ASTM Committee E-35.22.

9. Evaluation of results.

9.1 The remaining fish population may be evaluated in several ways. The most reliable method for small ponds is drainage of the entire basin, which virtually assures the capture of all remaining fish. Another reliable method is to treat the water again using a toxicant whose efficacy is well established. Enough time should be allowed between the treatments so the fish killed by the second cannot be confused with those killed by the original treatment. Methods for partial sampling of the remaining fish population are outlined in section 5.2.

9.2 Criteria of efficacy. Failure to capture live fish with extensive sampling effort is reasonable evidence (but never absolute assurance) of a complete kill. The post-treatment sampling should include two types of sampling gear. Refer to sections 5.2, 5.3 and 5.4 for sampling gear, methods of assessment, and sampling effort. A trial site which has not been drained or retreated with a known toxicant should be periodically observed for a year for evidence of remaining target fish.

10. Reporting.

10.1 Reports of field trials should include all pertinent data and detailed descriptions of methods in a format which permits ease of review.

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II. Amphibian toxicants and repellents.

Many repellents and toxicants for amphibians could be tested following the methods used for fish, since in most cases problems caused by them are associated with fish production. Tadpoles, for example, are no problem where there are bass present in ponds, but in hatchery situations they may multiply rapidly and interfere with the growth of desirable fish (Helms, 1967). Terrestrial amphibians have been documented as creating problems for bee keepers (Eckert, 1934).

Laboratory studies.

It is necessary to know the complete life history of the particular amphibian which one wishes to control, since the laboratory studies would vary according to the phase of the life cycle the toxicant or repellent is to control. Testing to determine LC50 value at 24, 48, and 72 hr. (Helms, 1967) was done in 15-gal aquaria. Three to 5 test animals were placed in each aquarium and then the test chemical was added. A sixth aquarium was maintained as a control. Bullfrog and leopard frog tadpoles and a few species of toad tadpoles were tested. Seven species of fish were also tested under identical conditions.

Field studies.

After testing in aquaria, Helms conducted field studies. The methodology varied depending on the life history of the organism. In the case of tadpoles, 9 treatments were carried out in 6 different ponds. Following treatment, the tadpole numbers were checked by seining to determine the completeness of kill and whether nontarget species were affected.

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A. Terrestrial amphibian and reptile toxicants and repellents.

Lizards seldom need control. Turtles can be controlled by trapping, fishing, or shooting (Dillard, 1973). The hunting or taking of alligators is regulated by Federal and State law. Poisonous snakes in various geographic areas may cause human death or injury and therefore may be identified as pests. Many snakes are beneficial or neutral because they consume rodents and insects. However, some reptiles may require control.

Toxicants. No test methods for reptile toxicants were identified for review.

Repellents. Some of the literature points out that repellents may be acceptable control measures. Cowles and Phelan (1958) have shown that snakes are very sensitive to chemical stimuli. Crawford and Gross (1969) have shown that snakes show heart and respiration rate responses to chemical stimuli.

Porter and Czaplicki (1974) have shown that both water snakes and garter snakes are sensitive to chemical cues produced by themselves or other species. The authors conducted a series of experiments to test the attractiveness or the repellency of snakes or others of the same species or other species. A method for screening potential snake repellents has been developed by Jenkins (1962).

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B. (Reserved).

III. Avian toxicants, repellents and reproductive inhibitors and anesthetizing chemicals.

No specific laboratory or field test protocols exist for the efficacy evaluation of avian toxicants, repellents, reproductive inhibitors and anesthetizing chemicals. Publications by Lee (1970), Ochs (1972), and Schafer and Guarino (1970) provide brief descriptions of, and the rationale behind, some tests. Since many of the laboratory tests necessary to establish efficacy are described in detail in Subdivisions E and F of these guidelines, this discussion will elaborate on laboratory tests only when it is necessary to provide additional information relative to avian control. The primary emphasis will be directed toward efficacy determinations in the field.

Discussions relative to laboratory studies for toxicants, repellents, reproductive inhibitors and anesthetizing chemicals have been combined under one heading ("laboratory methods"), since a majority of the laboratory tests must be conducted on all chemicals irrespective of the proposed use. References to special studies or special emphasis relating to individual proposed uses are made in the text.

Discussions relative to field studies are divided into four major groups, since evaluation procedures in the field vary considerably. Field test protocols for bird control agents are still in the developmental stages. Since many of the referenced tests were conducted before the most recent legislative action on FIFRA, as amended, most of them are lacking in one or more of the studies now required. A number of areas can and should be strengthened with regard to all tests:

1. Better knowledge of cultural practices and bird populations in test areas;

2. Use of larger experimental units;

3. Strict adherence to treatment schedules;

4. Use of controls during the same test period;

5. Use of more replications per test;

6. Proper application of statistical techniques; and

7. More adequate appraisal of hazards to nontarget species.

It must also be recognized that the field evaluation of efficacy of any bird control agent depends on conducting the test in the proper area at the proper time. Birds and biologists are not always able to mesh their activities in well controlled or ideal test situations; therefore, a proposed protocol must be adaptable to existing test situations.

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A. Avian toxicants, repellents, reproductive inhibitors and anesthetizing chemicals: Laboratory methods.

Acute toxicity.

The various methods for determining acute oral and dermal toxicity in birds are described in this Appendix; however, since control agents are seldom developed for use on the test species recommended in Part VII (i.e., ducks and quail), it will be necessary to conduct LD_{50} evaluations on the target species and on selected nontarget species which may be affected by the proposed control agent. The moving point interpolation method described by Thompson (1947), Thompson and Weil (1952), and Weil (1952) is particularly appropriate for this purpose. Descriptions of the use of this method for wild birds are provided by DeCino et al. (1966), Schafer (1972), Schafer et al. (1973), and Tucker and Crabtree (1970).

Subacute toxicity.

The exact technique used to determine subacute toxicity will depend on the proposed application of the control agent. Tests should be conducted on target and selected nontarget species. For proposed applications resulting in short-term exposure, the 5-day dietary LC_{50} test protocol developed by Heath and Stickel (1965) as modified by Hill (1972) may suffice. Since many applications of control agents (i.e., repellents) may result in longer exposure, longer term tests should be conducted to approximate the maximum period of exposure. The 7- to 40- day test procedures described by Schafer and Marking (1974), Schafer et al. (1975), and Tucker and Crabtree (1970) may suffice for most applications. It may, however, be necessary to conduct studies for as long as 90 days; in this case the methodology described by Hayes (1967) may be acceptable. Again, the size of test populations will necessarily vary with the availability of the test species.

Inhalation toxicity.

Data on inhalation toxicity will be necessary on the target species and on representative nontarget species if the proposed use of the control agent results in respirable gas, fumes, aerosols, or dusts. Because there are no published records of laboratory tests using wild avian species, modified mammalian tests should be used.

Secondary hazards.

Since the use of avian control agents in urban and suburban areas presents a potential hazard to carnivorous avian and mammalian predators, indicative data related to the proposed use should be gathered to define the degree of hazard to canines, felines, or mustelids, at least one species of hawk, and one species of scavenger bird. Schafer et al. (1974a) describes some test methods. Because of the nature of the tests and availability of suitable test animals, small test populations will be acceptable unless hazards are encountered. If hazards are found, more extensive testing may be required.

Repellents.

The repellency (or acceptance) of a compound can be determined by a number of different techniques. The initial evaluation may utilize the individual response-no choice method described by Star et al. (1964) and modified by Schafer and Brunton (1971). Although this method usually overestimates the amount of chemical needed to produce a repellent response under field conditions, it does provide data on the maximum treatment levels needed to produce protection. To determine comparative repellency (or acceptance) of a chemical in individual birds and groups the methodology described by Hill (1972), Luckwill and Weaver (1965), McLean (1972), or Ridsdale and Granett (1969) may be acceptable. Although indications of the repellency (or acceptance) of chemicals can also be obtained when the chemical is incorporated into drinking water (Duncan 1963), tests on foods are preferred unless water is the item to be protected.

Reproductive inhibitors.

Few laboratory methods have been published on wild avian reproduction. Since most wild birds are difficult to breed in captivity, domesticated species have generally been used. The application of a potential reproductive inhibitor to food is the principal treatment method, assuming the proposed application uses oral ingestion. Methododology for determining efficacy in domesticated species is described by Elder (1964), Powell (1966), and Wentworth (1970) for chronic feeding; Jones et al. (1972) for acute treatment of males; and Wentworth et al. (1968) for the treatment of eggs.

Anesthetizing Chemicals

Laboratory methods for testing a chemical's effectiveness as an anesthetizing chemical are described by Cline and Greenwood (1972), Peek (1966), Schafer et al. (1967), and Schafer and Cunningham (1972).

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B. Avian toxicants - Field methods.

There are numerous published methods that can be used to appraise the efficacy of oral bird toxicants under different application situations. Although the methodology described covers basic needs to determine efficacy, more effort needs to be expended with regard to nontarget species.

Acceptable test methodology for determining the efficacy of dermal toxicants applied as sprays is readily available: Jackson and Park (1973); Lefebvre and Seubert (1970); Marsh (1964); and U.S. Fish and Wildlife Service (1973). Although these methods may be used for spray applications, they are deficient in information regarding hazards to non-target species. A single test for contact toxicants is available (Schafer et al., 1969); again, additional data on hazard to non-target species may need to be provided.

A single published test is available for determining the efficacy of inhalation toxicants (Devore et al., 1966).

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C. Avian repellents: Field methods.

The determination of the field efficacy of avian repellents is based on 3 main factors--changes in behavior, reduction to bird numbers, and a reduction in damage. There are 2 major types of chemical repellents-gustatory and tactile; each requires somewhat different evaluation procedures.

The gustatory repellents (those acting through some physiological mechanism during or after oral ingestion) have been the most thoroughly investigated, and appraisals exist for sprouting agricultural seeds (Besser, 1973; Frank et al., 1970; Guarino and Forbes, 1970; Herman and Kolbe, 1971; Ingram et al., 1973; Stickley, and Guarino, 1972; West and Dunks, 1969; and West et al., 1969); sprouting horticultural seeds (Abbot, 1958; Royall and Ferguson, 1962); and ripening grain (DeHaven et al., 1973; Dolbeer et al., 1973; Guarino et al., 1973, 1974; and Stickley and Ingram, 1973). It is particularly important that minimum plot sizes be established according to the recommendations of Guarino (1972) and Herman and Kolbe (1971), and that replications be used.

Other gustatory repellents are those that induce a behavioral response in a very small proportion of birds causing damage. Four published appraisals contain information: Besser et al. (1973); DeGrazio et al. (1971, 1972); and Stickley et al. (1972). Again, test plot size and replications are of prime importance.

There are no published acceptable procedures for evaluating tactile avian repellents.

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D. Avian reproductive inhibitors: Field methods.

Only a few tests on efficacy of reproductive inhibitors have been conducted to date. The variety of species evaluated is limited and the adequacy of test methodology in individual studies is not established. Efficacy determinations require measuring fertility rates of treated and untreated birds and effects on breeding behavior, production, and population levels.

Test procedures have been developed for evaluating the effects of reproductive inhibitors in various blackbird species (Bray et al., 1974; Fringer and Granett, 1970; Messersmith, 1971; Peek, 1972; and Vandenbergh and Davis, 1962). In addition to the production studies common to each paper, histological evaluations of treated birds (male and/or female) are outlined by Vandenbergh and Davis (1962), and behavioral aspects are described by Bray et al. (1974) and Peek (1972). Effects on nontarget species should also be included in the test design.

Test procedures are less well developed for field evaluations in pigeons (Shortemeyer and Beckwith, 1970; Wofford and Elder, 1967; and Woulfe, 1968) and should be augmented with data on productivity, histology, breeding behavior, and effects on nontarget species.

Procedures are also available for gulls (Wetherbee, 1967).

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E. Avian anesthetizing chemicals: Field methods.

The most important measures of the field efficacy evaluation are percent capture, mortality, the time until anesthesia, and the duration of anesthesia. For control programs, population reduction by capture and removal is also important.

Methods for evaluating the effects of anesthetizing chemicals on upland game birds have been described by Mosby and Cantner (1956), Williams (1966, 1967), Williams and Phillips (1972), and Williams et al. (1966). However, additional information, such as hazards to nontarget species and body residues in treated birds, may also be required. Accurate scientific description of measurement parameters must also be used (i.e., g/kg bait, not g/cup).

Methods for using anesthetizing chemicals to capture waterfowl are also available (Crider and McDaniel, 1966, 1967, 1968, and Crider et al., 1968); however, these methods are not complete, and additional information may be necessary. Additional methodology is described for other species of birds commonly found near water (Smith, 1967 and Williams and Phillips, 1973).

Methodology for urban pigeon and sparrow control with anesthetics is well detailed by Murton et al. (1963), Ridpath et al. (1961) and Thearle et al. (1971), although certain aspects (e.g., hazards to domestic animals) may need more documentation. Methodology for the use of anesthetizing chemicals in rural situations is best described by Martin (1967), Murton et al. (1963), Ridpath et al. (1961), and Williams and Phillips (1972); however, hazards to predatory animals may need more documentation.

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IV. Bat toxicants and repellents.

Bats are beneficial in the United States in that they consume great quantities of insects. Bats can present local nuisances, however, when they inhabit the attics of private homes or the rafters of public buildings. Because bats can be vectors of rabies, chemical bat control may become necessary in circumstances where the likelihood of bat-human contact is high. The goal of bat control, whether by toxicant or by repellent, is the elimination of the problem. Bat control with pesticides in buildings should reduce the target populations to near complete eradication. Products with marginal efficacy are essentially useless in solving bat problems. Therefore, products should produce 90% control or better in efficacy tests.

To demonstrate efficacy, it is necessary to develop a reliable means of estimating the number of bats in the target area or, at the very least, of assessing the level of bat activity. Direct counts of bats leaving premises have proven to be useful (Greenhall and Stell, 1960). When using this method, it is necessary that all openings used by bats be visible at all times. A team of observers may be required. If the bats use few points of entry, it may be possible to monitor activity by use of photoelectric cells which activate electical counting devices. Such devices would not differentiate between bats entering and bats leaving, and could, therefore, provide only relative activity indices.

Through thorough exploration of infested areas by day, it may be possible to make absolute counts of roosting bats. The size of the infestation could also be assessed through droppings counts. However, these activities may disturb the bats, causing them to return in fewer numbers even if no bat control agent is applied. Physical disturbances due to product application may also exert repellent effects. Check studies (with no chemical applied) are therefore necessary to establish the efficacy of the chemical agent itself. Care must be taken that physical disturbances in check and treatment studies be equivalent.

The list of references provided below includes material on the biology of bats, the trapping of bats, the maintenance of bats in captivity, bat control, bats as rabies vectors, and other information which may be useful in developing products and control strategies.

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V. Rodenticides: commensal and non-commensal.

One of the earliest--and relatively comprehensive--publications available on rodenticides and their efficacy is "Biological Methods for the Evaluation of Rodenticides" (Bentley, 1958). In 1963, Kverno and Hood published a volume on the evaluation procedures and standards for screening and developing chemicals for forest rodent control.

Tracking powders came into use almost by accident when deaths among house mice were observed in places where insecticides such as DDT and BHC were used. Until its ban in 1972, DDT was used quite extensively for house mouse control. Anticoagulant rodentcides were found effective as tracking powders for both rats and mice and, until recently, were used more in Europe than in the United States.

Little is available on methods for evaluating tracking powders. Bentley (1958) devotes a section of his bulletin to tracking powders, with reference to their evaluation. Szuber and Brodniewicz (1964) provide a drawing of their test cage and the results achieved with some some anticoagulant tracking powders. More recently, Tanaka (1973) published evaluations of rodenticides as tracking powders, and described the methods utilized in his studies.

Laboratory methods. The Environmental Protection Agency has developed methods that have been used for establishing laboratory efficacy of rodenticides for commensal rodents (See Exhibits 2-20).

Articles concerning laboratory test methodology have been published by various researchers: World Health Organization, 1967; Bentley and Larthe, 1959; Dicke and Richter, 1946; Durbin and Robens, 1964; Emlen and Strecker, 1951; Hayes and Gaines, 1959; Hankins et al., 1973; Howard et al., 1968; Loosjes, 1959, Palmateer, 1974, 1977; Saunders, 1955; Savarie et al., 1973; and Ward et al., 1949.

Field methods: Emlen and Crow (1951), Miller (1953), Richens (1967), Schein (1950), and Wood (1965) have reported on field studies which utilized techniques or methodology of value to those unfamiliar with setting up field evaluations.

The food preferences of the target species, as well as the acceptability or palatability of the prepared bait will have a direct bearing on efficiency in both the laboratory and field. Palmateer and McCann (1976) found a very strong relationship between acceptance of anticoagulant rodenticides and mortality. Thus, techniques or methods for measuring food preferences or acceptance should be recognized as an integral part of evaluating a rodenticide for any species. Bentley (1958) discussed palatability of baits in considerable detail. His evaluations included measuring the number of visits to the bait, duration of feeding, amount consumed, and rate of intake. He also included comments on the time of onset of poison symptoms and bait shyness.

Shumake et al. (1971) made comparisons of taste preference studies between laboratory and wild Norway rats. Barnett and Spencer (1953) conducted an extensive study of food preferences of wild Norway rats, and Khan (1974) conducted laboratory experiments on food preferences of the black rat. Other researchers, such as Gregson (1966), Cornwell and Bull (1967), Beer (1964), Patric (1970), Fitch (1954), Joy et al. (1967), and Nass and Hood (1969) have published data which may be valuable to those trying to formulate better rodent baits.

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A. EXHIBITS 2-20

(I.) The methods listed below [see (III.)] are published by the Agency in its "Manual of Biological Testing Methods for Pesticides and Devices, Volume 1." Copies of individual test methods may be obtained by writing to the Environmental Protection Agency, EPA Chemical and Biological Investigations Branch, Agric. Res. Center, Bldg. 402, Beltsville, Md. 20705, or Product Manager (16), Insecticide-Rodenticide Branch, (TS-767), Environmental Protection Agency, 401 M St., S.W., Washington, D.C. 20460.

Users are advised that some BSFD (Benefit and Field Studies Division) methods listed below are undergoing review and possible revision. Excerpts from Exhibits 2 to 20 are provided in (II.) below. These excerpts call attention to important specific testing conditions and requirements, such as caging, challenge diets, selection of test animals, environmental conditions, and specific requirements for particular uses.

(II.) Test standards.

(A.) Type, weight, and number of animals.

1. For a new claim use the wild target species. These may be wild-caught or from a wild colony. For an existing claim use either the wild target species or laboratory strains of the target species. The Wistar is the preferred strain of the laboratory rat, and the Swiss-Webster is the preferred strain of the laboratory mouse. Roof rat claims may be supported by Norway rat data. Baits presented to a species in an unopened place pack must have a test on the formulation itself and on the place pack.

2. All animals shall be outwardly healthy, active, sexually mature, and fall within the following weight classes (in grams):

	Minimum	Maximum	Max. Acceptable Differences In Mean Wts. between Sexes
Lab rats	150	300	50
Norway rats	150	400	65
Lab mice	15	35	5
House mice	10	25	3

3. The test should be replicated at least once. For each test expose at least 10 males and 10 females to the toxicant. Use another 10 males and 10 females as controls. If a series of tests is conducted concurrently, use at least one control group.

(B.) General environmental conditioning.

1. All animals used in these tests must be held for observation in the laboratory for one to four weeks prior to testing. Ectoparasite control with appropriate concentrations of carbaryl, malathion, or pyrethrum dusts is permissible if applied externally to both test and control animals not less than seven days prior to testing. Acclimate animals to test conditions three days prior to testing. Animals should not be subjected to undue or unnecessary stress from noise or human activities (e.g., movement). Human activity within the animal test room should be minimal. Water and commercial rat or mouse diet must be available to them at all times. Do not use the standard rodent challenge diet described below for pre-test feeding.

2. During holding and test periods the animals should be held in areas with the following environmental conditions.

Temperature	20 to 25°C. Strong air currents from heaters or air conditioners shall not blow directly onto the animals.
Relative humidity	50 to 55%
Light	12 hrs artificial light per day, not to exceed 2153 lx (200 ft candles) at cage location. Total reversing of natural photoperiods is not recommended.

(C.) Standard rodent challenge diet.

1. A standard rodent challenge diet must be provided whenever the animals are exposed to a toxic bait. This challenge diet shall be composed (by weight) of 65% cornmeal (whole yellow ground corn), 25% rolled oat groats (ground), 5% sugar (10X powdered or confectioners, 95+% purity), and 5% corn oil (95+% purity). Combine dry ingredients together, add oil, and thoroughly mix. Be certain that the mixing utensils are free of contamination before preparing the diet.

2. The whole (not degerminated) yellow ground corn shall be from the most recently available crop and be fresh ground. Seventy-five percent (± 5%) shall pass through a #10 screen (10 meshes to the inch or 2.54 cm) and 50% (± 10%) be retained by a #20 screen (20 meshes to the inch). The oats shall be steam rolled oat groats (oat seeds with hulls removed) and coarsely ground after the rolling process. Seventy-five percent (± 5%) of the ground oats shall pass through a #5 screen (5 meshes to the inch) and 50% (± 10%) be retained by a #20 screen. The corn oil shall be of the type available as cooking oil, undiluted with other oils, and shall not be rancid.

3. The challenge diet shall be stored under refrigeration if it is to be used within three days of preparation. If it is to be held for longer periods, the diet shall be packaged in plastic containers [2.2 to 4.5 kg (5 to 10 lb) per container], tightly closed or sealed, and maintained at -18°C or below until used. It shall be at room temperature when offered to test or control animals. Mixed challenge diet shall not be stored longer than six months.

(D.) Specific conditions for dry baits, wax blocks, and wax pellets.

1. For rats, place animals in individual cages with a minimum bottom surface area of 0.538-2.15 sq ft (500-2000 sq cm). For mice, place animals in a group cage with a minimum bottom surface area of 1.61-2.15 sq ft (1500-2000 sq cm). For mouse shelters, use empty cans with one end removed and slightly flattened to prevent rolling. Use one can per five mice. Provide shelters in both the test and control cages.

2. The rodenticide-treated food and the standard rodent challenge diet are each offered to the test group in separate cups in excess of the daily food requirements. The control group is offered only the rodent challenge diet. The gross weight of each cup and its contents is determined daily and returned to the starting weight by addition of food. If food becomes fouled by urine or feces, replace food in each cup. Each day record the quantity of food consumed during the preceding 24 hours. Spilled food should be recovered for weighing to establish exact food consumption. Where food spillage is damp, it shall be dried to its original moisture content before weighing. The positions of the bait and challenge diet cups are reversed every 24 hours to counter any feeding position preference of the test animals.

(E.) Specific conditions for liquid baits.

1. For rats and mice, group cage animals in solid-bottomed all-metal cages, having a bottom surface area of 18.3-26.9 sq ft (17,000 to 25,000 sq cm. The bottom of each cage should be covered to a depth of approximately 1 inch (2.5 cm) with clean wood shavings. Each cage should contain two metal nest boxes, approximately 14 by 14 by 4 inches (36 by 36 by 10 cm) without bottoms.

2. Use metal or ceramic feeders designed to prevent spillage or contamination of the diet. Provide each cage with two or more feeders, filled daily with fresh challenge diet to provide at least 40 grams per rat or 2 grams per mouse.

3. Use graduated 100 ml no-drip waterers fitted with ball-type watering tubes. Do not use automatic or open-cup type waterers. Provide each treated test group with a minimum of 12 waterers for rats or 4 for mice. For a liquid bait, an alternate, non-toxic water source must be provided. Fill one half of the waterers with tap water and the others with test liquid diluted according to use directions. This arrangement provides each rat with 30 ml or each mouse with 10 ml of both liquids, which are their daily requirements. Replenish both liquids as necessary so that each waterer never becomes less than one-third empty. Record daily the total quantity of each liquid consumed during the preceding 24 hours for both the test and control groups.

(F.) Specific conditions for tracking powders.

1. For rats an acceptable test apparatus consists of two 150 gal (568 liter) stock watering tanks connected by two square hardware cloth tunnels 36 inches (914 mm) long with 4-1/2 inch (114 mm) sides, top, and bottom. The tunnels are parallel and spaced 40 inches (1016 mm) apart. A galvanized steel removable pan 12 inches (305 mm) long by 4 inches (102 mm) wide with a 1/4 inch (6 mm) lip on both sides is centered in each tunnel. The test animals must have free and equal access to both tanks through the tunnels. In each tank place bowls of the standard rodent challenge 613 diet and water *ad libitum*. Each tank contains a screened-bottom nest box approximately 14 by 14 by 4 inches (35 by 10 cm).

2. For mice an acceptable test apparatus consists of two screened-bottom 15 by 18 by 9 inches (381 by 457 by 229 mm) cages connected by two square hardware cloth tunnels, 36 inches (914 mm) long with 4-1/2 inch (115 mm) sides, top, and bottom. The tunnels are parallel and spaced 4 inches (102 mm) apart. A galvanized steel removable pan 12 inches (305 mm) long by 4-1/2 inches (114 mm) wide with 1/4 inch (6 mm) lip on both sides is centered in each tunnel. The test animals must have free and equal access to both cages through the tunnels. In each cage place bowls of the standard rodent challenge diet and water *ad libitum*. Provide shelters in both cages. Use empty cans with one end removed and slightly flattened to prevent rolling. Use one can per five mice.

3. Place ten male and ten female mice or rats in the appropriate testing apparatus. Five days after the introduction, dust one of the removable steel pans with the tracking powder according to label dose rates (weight per surface area). Any additional dust must be applied only to this pan.

4. For tracking powder, the animals is always provided with a non-toxic route to all sources of food and water. Maintain a daily record of the degree of activity across the dust on the treated plate. All previous signs of activity are covered after the daily reading.

(G.) Exposure periods. The exposure period for a single-dose toxicant is 3 days. For multiple-dose toxicant, the exposure period is 15 days.

(III.) List of Exhibits.

Exhibit 2 (TSD # 1.201) - Standard Norway rat anticoagulant liquid bait laboratory test method.

Exhibit 3 (TSD # 1.202) - Standard house mouse anticoagulant liquid bait laboratory test method.

Exhibit 4 (TSD # 1.203) - Standard Norway rat anticoagulant dry bait laboratory test method.

Exhibit 5 (TSD # 1.204) - Standard house mouse anticoagulant dry bait laboratory test method.

Exhibit 6 (TSD # 1.207) - Standard Norway rat acute liquid bait laboratory test method.

Exhibit 7 (TSD # 1.208) - Standard house mouse acute liquid bait laboratory test method.

Exhibit 8 (TSD # 1.209) - Standard Norway rat acute dry bait laboratory test method.

Exhibit 9 (TSD # 1.210) - Standard house mouse acute dry bait laboratory test method.

Exhibit 10 (TSD # 1.213) - Standard Norway rat anticoagulant wax block and wax pellet laboratory test method.

Exhibit 11 (TSD # 1.214) - Standard house mouse anticoagulant wax block and wax pellet laboratory test method.

Exhibit 12 (TSD # 1.215) - Standard *Peromyscus* species acute dry bait laboratory test method.

Exhibit 13 (TSD # 1.216) - Standard *Peromyscus* species anticoagulant dry bait laboratory test method.

Exhibit 14 (TSD # 1.219) - Standard rat acute place-pack dry bait laboratory test method.

Exhibit 15 (TSD # 1.220) - Standard mouse acute place-pack dry bait laboratory test method.

Exhibit 16 (TSD # 1.217) - Standard rat anticoagulant place-pack dry bait laboratory test method.

Exhibit 17 (TSD # 1.218) - Standard mouse anticoagulant place-pack dry bait laboratory test method.

Exhibit 18 (TSD # 1.205) - Standard Norway rat anticoagulant tracking powder efficacy laboratory test method.

Exhibit 19 (TSD # 1.212) - Standard house mouse anticoagulant tracking powder efficacy laboratory test method.

Exhibit 20 (TSD # 1.211) - Standard Norway rat acute tracking powder efficacy laboratory test method.

D. Rodent fumigants.

The information on the test methods or procedures for evaluating fumigants (toxic gases) for rodent control is sparse.

Laboratory Methods.

It may be necessary to draw on some of the laboratory procedures used by entomologists or to rely on the methods used to evaluate euthanasia materials for laboratory animals and unwanted cats and dogs. Glen and Scott (1973), for example, describe a simple chamber for the use of carbon dioxide as a means of euthanasia for cats; Smith et al. (1973) describe an apparatus for anesthetizing small laboratory rodents which could be readily adapted to evaluate rodent fumigants under laboratory conditions.

Field Methods.

A field test used to evaluate methyl bromide as a fumigant for ground squirrels (Berry, 1938) provides a basis for designing field test methods. A rather comprehensive study on the effects of various fumigants on fleas was conducted by Steward and Mackie (1938). A woodchuck control experiment was carried out by de Vos and Merrill (1957) using gas cartridges. Some early experiments with calcium cyanide on ground squirrels by Sanders (1921) provide some useful field evaluation techniques. Miller (1954) used the open hole test to evaluate a number of potential fumigants for pocket gophers. In the course of research on a method of fumigation using a rodenticide cartridge, simulated natural burrow systems were constructed and tested by Fuhr and Silver (1947).

A test design is needed which can be used to evaluate fumigants in the laboratory and which would be a suitable technique to use for all rodent species that may be controlled with fumigants. Field evaluations will differ widely depending on the species, habitat and intended use practices.

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de Vos, A., and H.A. Merrill. 1957. Results of a woodchuck control experiment. J. Wildl. Manage. 21(4):454-456.

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C. Rodent repellents.

Although few repellents are presently used in rodent control, much research was undertaken in the past. Examples of some of the test methods used in evaluating some rodent repellents are discussed; others are cited in the literature. Since repellents encompass such a wide range of effects, a complete coverage is not easily attained and has not been attempted here.

Repellents are generally classed by their intended use, the most common being to protect growing crops, forest or fruit trees, or ornamentals. Other repellents have been developed for coating seeds to protect them from rodent depredation. Further, a great deal of effort has been expended on repellents for commensal rats and mice to minimize damage to food packages, textiles, and other materials in storage and shipment, and to building materials and cables. There are also chemicals which have been explored as area repellents to keep rodents from entering and/or occupying certain areas.

1. Rodent repellents for protecting living plants.

Although repellents have been explored over many years to protect growing crops, forest or fruit trees, and ornamentals from various rodents such as squirrels, porcupines, rabbits, and hares, few test methods have been reported in the literature.

Cardinell and Hayne (1947) describe field tests for rabbit repellents using treated twigs and apple trees. The experiments were planned to take advantage of the analysis of variance methods of statistical comparison. Later, Hayne (1949) described both pen and field tests for protecting gardens from cottontail rabbits. Hooven (1966) provided a technique for evaluating a repellent to protect Douglas fir trees from hares and rabbits.

Dodge et al. (1967) described an experiment for use under semi-field conditions to test deer and rabbit repellents on trees. They utilized a randomized complete block design and analyzed the data by analysis of variance.

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Hooven, E.F. 1966. A test of thiram on two rabbit-infested areas of Oregon. Tree Planter's Notes 79:3.

2. Rodent repellents for protecting seed.

Laboratory methods.

Kverno and Hood (1963) published a method of screening seed protectants. Their testing period, however, was only 3 days, which may have excluded the discovery of many aversive conditioning repellents. Lindsey et al. (1974) conducted laboratory tests on the repellency of Douglas fir seeds coated with mestranol. He expressed the amount of treated seeds consumed as a percentage of the untreated seeds eaten. Passof et al. (1974) used the percentage of treated seeds cracked open as an index of repellency for deer mice in laboratory tests; less preferred alternate food was provided to prevent starvation, should the mice be highly repelled by the treated seeds. Radvanyi (1970) expressed repellency as "percent effectiveness" and calculated his efficacy using the following formula:

$$\frac{\text{\% untreated seeds destroyed} - \text{\% treated seeds destroyed}}{\text{\% untreated seeds destroyed}}$$

Field methods.

In field studies of conifer seed repellents, the repellency is often measured indirectly by comparing the number of seedlings produced in an area seeded with treated seeds to that of an area seeded with untreated seeds (Passof et al., 1974; Lindsey et al., 1974).

Roy (1961) used a seed spot technique to test a specific seed protectant under field conditions. The difference in the percentage of treated and untreated seed that survived was used to evaluate the results.

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3. Rodent repellents for protecting packaging materials.

Just preceding World War II, considerable effort was exerted to develop commensal rodent repellents or deterrents for the protection of paper and cardboard packing materials. A number of laboratory test methods was developed for screening and evaluating chemicals for repellency.

Bellack and DeWitt (1949) published one test method which was used extensively in early evaluations. This test used laboratory rats housed in individual cages and offered 2 food cups: one with a standard laboratory chow and the other with the laboratory chow containing the candidate repellent. The test period was 4 days, and consumption from each cup was recorded daily. Bellack and DeWitt (1949) developed an arithmetic expression that yielded a numerical value for repellency of treated food items. Their test procedure took into account physiological effects, random feeding, and variations of animal weights. Welch et al. (1950) used the same technique and compounds.

Feeding tests became a useful procedure for rapidly screening potential repellents. Barnett and Spencer (1953) used a slight variation of the usual feeding studies by placing the deterrent below the food item, thereby attempting to measure the influence of odor alone as a repellent.

The exposure of chemically-treated materials (i.e., containers, etc.) in large enclosures containing rat populations has been used by a number of researchers (DeWitt et al., 1950; Welch, 1951; Welch and Duggan, 1952; Welch, 1954).

Stolurow (1948a) described a laboratory testing device called the Pittsburgh Obstruction Unit. He used this unit to evaluate the effectiveness of repellents in preventing the destruction of barriers. The basic principles of this method have been used in simpler and less expensive designs for testing repellents on barriers (Bellack and DeWitt, 1950). Stolurow (1948b) prepared a laboratory method for the evaluation of rodent deterrents.

Analysis of the potential repellency of various packaging and structural materials is discussed by Weeks (1959). Rats are offered food on the opposite side of a barrier and are timed to determine how long it takes them to gnaw through. The time required for barriers treated with the test repellent is compared to that required for untreated control barriers. Weeks (1959) developed another repellent test called the graded strip test. A strip of corrugated paperboard is placed over a row of peanuts so that the rat must gnaw through the board to get to the peanuts. The further up the strip the rat gnaws, the less the effectiveness of the repellent. Statistical evaluation was based on the method of biological assay for graded responses used by Burn et al. (1950).

Tigner and Besser (1962) devised a new quantitative method of evaluating chemicals as rodent repellents on packing materials. Tigner (1966) later reported on a series of compounds used to treat multiwall tarps and bags. He devised a qualitative method of evaluating the chemicals as potential rodent repellents.

Bull (1972) describes an approach to evaluating attractants and the influence different odors have in inducing Norway rats to feed at given locations. In this study the effect of certain repellents was also examined.

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4. Rodent repellents for protecting wire and cable coatings.

Anthony and Tigner (1967) briefly describe both laboratory and field tests of a repellent used in various types of communication wire or cables. Field studies involved a number of geographical areas, which was essential because of the different rodent species involved. Also, environmental conditions associated with severe cable damage vary from region to region.

Howard (1953) conducted laboratory tests of pocket gophers gnawing electric cables. His test method confined pocket gophers to one part of a cage; a short section of cable was positioned and fastened to block the passageway to the remainder of the cage. By this means data could be obtained for treated and untreated cables and for cables with different types of covering or insulations.

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D. Rodent reproductive inhibitors.

A reproductive inhibitor can be defined as an agent that can cause permanent or temporary sterility in either or both sexes, or, through some other physiological mechanism, reduce the number of offspring or alter the fecundity of the offspring produced.

The numerous compounds capable of inhibiting reproduction in various rodent species have diverse and complex modes of action (Jackson, 1959; Saunders, 1968). Many of these compounds do not lend themselves to standard laboratory or field evaluation methods. Both Jackson and Saunders provide reviews of the capabilities of various compounds.

Because of the intensive search for oral contraceptives for humans, a large number of references are available. The literature cited illustrates representative methods of measuring the physiological effects of compounds on inhibiting reproduction in rodents commonly used in the laboratory.

Laboratory methods.

Histological evaluations of the effects of chemicals on cellular modifications or makeup of reproductive systems of rodents is a procedure used in some laboratories. However, histological evaluation is laborious and expensive when conducting preliminary testing or screening new compounds (Hershberger et al., 1969). Studies of the testes in treated rats are examples of this method in determining the effects of certain chemosterilants (Pate and Hays, 1968; Ericsson, 1970; Ericsson and Baker, 1970; Gibson et al., 1967).

Many researchers have used the number of pregnant females, implantations and/or the number of rats that ovulated to determine the success of several orally active estrogens and progestins (Kincl and Dorfman, 1965). Banik et al. (1969) made a statistical analysis of the data from the females with ova or implantations using the four-fold contingency test tables of Mainland and Murray (1952). The average number of ova or implantation sites in each rat in the experimental series was compared with those of the controls.

Boris et al. (1971) demonstrated a method which may have merit in establishing more precise effects of compounds on ovulation by using the pubertal ovulation in the rat for the study of anti-ovulatory compounds. Uterotrophic responses as measured by the mean uterine ratio (milligrams of uterus per gram of body weight) is a means of expressing the estrogenicity of certain potential chemosterilants (Rooks et al., 1968; Saunders and Rorig, 1964).

Efficacy of potential rodent chemosterilants can be analyzed in laboratories by using some of the following indices: average number of days from mating to the appearance of first litters; average total offspring produced per female; average number of litters produced per female (Generoso et al., 1971; Howard and Marsh, 1969). Survival of the offspring and their reproductive capabilities may also be used to determine efficacy expressed in the F_1 generation (Kinel et al., 1965; Clancy and Edgren, 1968). These are only some of the commonly used criteria for efficacy.

Where a compound may affect spermatogenesis, efficacy of that compound may be determined by using a serial mating method. This technique involves placing the treated male with 1 or 2 females for a prescribed number of days and then moving him on to new females for a similar period. Thus, by pairing the males with a series of females, it is possible to determine the time period in which sterility occurred and its duration (Hershberger et al., 1969).

Field tests (simulated).

Tests of a rodent chemosterilant may be conducted in large pens where small populations can be maintained and the tests can be replicated (Kendle et al., 1973; Gwynn, 1972a). Studies should be replicated a number of times under conditions where evaluations of effects can be exacting.

Field methods.

While all the methods cited have value, some may be more applicable than others. The characteristics of the compound, the proposed site, the intended pest, and the method of application will all influence the selection of the proper method.

Marsh and Howard (1969) evaluated mestranol as a reproductive inhibitor in Norway rats in garbage dumps. Two dumps were treated and a third served as a control. The age structure of the rat population and popuuation numbers were the criteria used to determine efficacy. Bowerman and Brooks (1971) carried out pen and field efficacy studies on Norway rats using quinestrol. Gwynn (1972a, 1972b) published pen and field results using still a different rodent chemosterilant.

These represent a portion of field studies which have been conducted. Field evaluations differ slightly from one another to meet the existing situations and the intended uses of the test compounds.

Significant reproduction inhibition assumes that comparable untreated populations are used as conrols to permit a sound statistical analysis. The Chi square analysis of the two-way analysis of variance might be the appropriate measure or the significant differences between the treated and control groups. Efficacy tests should be replicated to demonstrate a high degree of reproducibility results under various conditions of use. Potential chemosterilants also should be tested in widely separated geographical areas. Because evidence exists that many potential chemosterilants can be quite specific in their effects (Skinner, 1968), all species on which the product is to be used must be tested both in the laboratory and in the field.

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VI. Mammalian predacides, repellents, reproductive inhibitors, and devices.

Research in predator damage control has only recently attempted to provide information on the extent of predation and possible alternative solutions to past and present control methods. Balser (1974a, 1974b) has reviewed the status of coyote control, data on livestock losses, and research related to control methodology. His review and others (Cummings, 1966; Howard, 1974; Knowlton, 1972) have pointed to the paucity of data necessary for predator management. Methods without undesirable characteristics have not yet been developed.

Changing land-use patterns and land development have affected the habitats and food choices available to both predator and prey animals. Consequently, maintenance of wild animal populations above levels sustained by available natural foods encourages predation on domestic livestock. Control of animal damage to protect habitats, other wild species, and domestic products is basic to sound management and includes regulation of game and non-game populations. It is an essential element in carnivore management and must be considered in the light of current and developing land-use patterns.

Among the larger carnivores that cause damage and resulting management problems are the families Canidae, Felidae, Ursidae, and Mustelidae. Bobcats (Lynx rufus) may prey extensively at times on domestic animals and lynx (Lynx canadensis) may occasionally cause damage, but offending animals usually can be managed by mechanical methods. Bears (Ursus spp.) and mountain lions (Felis concolor) may cause extensive damage at times but are typically considered game animals. Again, offending animals are managed by mechanical methods sanctioned by State wildlife agencies.

The carnivores of primary interest here are coyotes (Canis latrans), gray foxes (Urocyon cinereoargenteus), red foxes (Vulpes fulva), feral dogs (Canis familiaris and various hybrids). They are particularly difficult to manage because of their inherent caution, adaptability, and high reproductive rates.

Evaluation of the efficacy of various control methods is extremely difficult because of the wide variation in circumstances where controls are applied. Heavily populated areas with small farms present a markedly different environment than open range with large land holdings and few humans. Climate and weather, habitat types, predator populations, reproductive rates, food bases, and feeding habits are some of the variables that often prevent replication of experiments under similar conditions. Another major impediment is the lack of adequate census techniques to measure populations and the changes induced by natural and applied controls.

Development of efficacy criteria for control methods will require cognizance of time and cost factors caused by the complex nature of predator-prey ecology and the extreme difficulty in securing uniform replicate field tests. Several years of study over extensive geographic areas under widely-varied climatic conditions will be needed to provide even minimal knowledge of predator-prey economics, behavior, and ecology. A review of information and data from field studies and other sources, such as consensus standards (e.g., American Society for Testing and Materials) may provide adequate guidelines for protection of nontarget species and the environment.

To summarize, effective canid control methods which meet all desired criteria do not exist. Known methods applied to minimize undesirable effects may provide efficacy data and essential damage control. Effective and useful methods vary with time and circumstance and may require removal of specific offending animals or local population reduction to achieve adequate depredation control.

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A. Mammalian predacides.

Laboratory methods for evaluating the toxicology and mode of action of candidate chemicals are well established and will not be considered here except as related to mode of application. Consensus standards developed by the American Society for Testing and Materials may provide additional guidance in evaluation of laboratory efficacy.

Atzert (1971), Crabtree (1962), Robinson (1970), Ward and Garlough (1936), and Ward and Spencer (1947), have summarized the toxicology and past use patterns of toxic chemicals to control canids. Field research data are limited. Experimental efficacy studies of the M-44 (sodium cyanide spring-loaded ejector mechanism) have been developed in several western states to evaluate its use in management of red foxes and coyotes. As a result of the efficacy studies, the Environmental Protection Agency has approved registrations for use of the sodium cyanide M-44 device.

No specific complete test protocols exist for field evaluation of toxic chemicals for predator management. Survey research data related to domestic livestock losses and relative effects of toxic baiting on coyote populations have been reported by Robinson (1948, 1953a, 1953b, 1961) and by Linhart and Robinson (1972). Effects on target and nontarget species populations are evaluated from trap-catch data and observations. Suggested methods for baiting to reduce hazards to nontarget species are given. The use of more than one control method in survey areas confounds the evaluation of a single method. The scope of the reports does provide extremely useful information on the effects of control and animal population trends over a time span.

Arrington and Edwards (1951) employed antelope population surveys to evaluate effectiveness of predator reduction on antelope productivity. Toxic chemicals were the primary reduction method, but overlapping use of more than one chemical, as well as other methods, prevented isolation of the effects of each method. Coyote population reduction was reported to result in greatly increased antelope productivity; however, coyote populations were not indexed.

Beasom (1973a,b) discussed relative effectiveness and selectivity of various methods of predator reduction to enhance wild turkey and deer productivity. High selectivity by the M-44 was noted.

Balser et al. (1968) reported results of a well-designed field study of predator reduction and waterfowl nesting success. Several reduction methods were used. Selectivity was not thoroughly discussed, but a similar design would be useful in evaluating specific methods if experimental areas were adequate.

Field tests of baits must consider food preference and availability to target species. Bait acceptance by extremely wary individuals or species usually requires that the chemical either be masked or be relatively tasteless. Also seasons, weather, temperature, and other factors affect bait acceptance.

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B. Mammalian predator repellents.

Few studies of potential repellents tested on wild carnivores have been reported. Definitive results from laboratory and pen tests are difficult to obtain and are not necessarily representative of behavior in wild free-ranging species under field conditions. In addition, highly variable physical and biological factors may obscure the real effects of potential repellents. Preliminary screening of methods with domestic animals may be useful before evaluation with wild species and field tests, if such limitations are recognized.

Nesse (1971), and Swanson and Scott (1973), reported results of limited field trails of the "Gerhardi Livestock Protector" to protect goats and sheep from coyote depredation, but replications were not adequate for accurate evaluation. Pfost (1971) conducted field trials of the Gerhardi device to protect weeder geese from dogs and coyotes. Replications were not uniform, but the study was reasonably well designed.

Gustavson et al. (1974) attempted to induce conditioned aversion in coyotes with lithium chloride; the discussion is not adequate for thorough evaluation. Jankovsky et al. (1974) reported briefly on the results of coyote repellent research in Colorado. Again, discussion of methodology and results is incomplete, and no wildlife census techniques were employed.

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C. Mammalian predator reproductive inhibitors.

Few studies have been directed toward management of wild canids by controlling reproduction. A wealth of information has been accumulated for screening tests of potential human contraceptives. These studies and others carried out with rodents, lagomorphs, dogs, and cats provide a reference source. Preliminary testing on domestic species to clarify physiological effects may be necessary because of difficulty in securing normal reproductive cycles in captive wild species. Pen tests with target species are essential, since separation of physiological effects from those caused by inadequate application techniques and other factors is extremely difficult with highly mobile wild species under field conditions.

Branker (1943) and Jackson (1953) have demonstrated clinical use of diethylstilbestrol (DES) to manage canine mismating. Kennelly (1969) determined abortifacient effects of mestranol in dogs during early gestation. Modification of Kennelly's procedure to determine effects at specific stages in the reproductive cycle is feasible. Balser (1964b) reported abortion in captive coyotes induced by a single oral dose of DES.

Modification of test designs employed by Linhart and Enders (1964) and Storm and Sanderson (1969) to determine reproductive inhibition in captive female foxes is necessary to delineate specific states of reproduction affected. Additional changes are needed to evaluate chemical effects on spermatogenesis and male fox fertility.

Linhart (1964b) reported results of field tests to determine bait acceptance by wild foxes, but the test design does not provide population measurements. Oleyar and McGinnes (1974) conducted field trials of DES to reduce fox reproduction.

Balser (1964b) discussed results of field trials with DES to reduce coyote reproduction. Linhart et al. (1968) reported results of the field trials by Balser (1964) and several others to determine effects of DES on reproduction of wild coyote populations. Inclusion of several trials with the variation in methodology employed prohibits a thorough evaluation. The authors suggest modifications of techniques and application but do not recommend specific methods.

Improved techniques for application of reproductive inhibitors and methods to measure populations are needed. In many wild species, relative indices may be the only feasible method to determine population levels and changes.

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D. Mammalian predator control devices.

The Agency does not register devices *per se*. Devices are registered when they are used to deliver a pesticide to a target animal (e.g., the use of the M-44 to deliver sodium cyanide to coyotes). Published research reports on control devices are few. Extensive overlap with toxic chemicals occurs since chemicals are employed in mechanical devices. Devices are also often used simultaneously with other methods. Separation of effects, therefore, is difficult.

Beasom (1973a,b) presents data and discusses the use of the M-44, shooting, steel traps, and strychnine baits to remove predators and enhance productivity of wild turkeys and white-tailed deer in specific study areas. Simultaneous use of several methods reduces the value of the data for determining effectiveness and selectivity of the various methods. However, high selectivity was noted for the M-44 and shooting when compared to traps and baits. Predator track counts and rodent snap trap transects were utilized to establish relative population indices, but specific census techniques were not provided.

Robinson (1943) discussed the use of steel traps and the "Humane Coyote Getter" for predator control. The report compares efficiency and selectivity, seasonal and weather effects, age of animals removed, experimental areas, and defects of the methods. Census techniques are not provided except indirectly by indices based on trap catch data. Hey (1964) provided a limited amount of data regarding use and selectivity of the "Humane Coyote Getter" in comparison with other control methods, and discussed hazards to nontarget species. No census or index methods are discussed.

Robinson (1953, 1961) and Linhart and Robinson (1972) provide data indication selectivity of steel traps in relation to species populations, and discuss population trends of target and nontarget species in predator control areas. Population indices are taken from trap-catch data. These reports do not provide the protocols, but they include highly useful information for developing test methodology.

Field methods reported by Balser et al. (1968) require adequate experimental area and population indices.

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E. Domestic dog and cat repellents and reproductive inhibitors.

The number of protocols in the literature for tests of repellency with dogs and cats is limited. A comprehensive search would require consultation with the workers in this field.

Repellents.

Huebner and Morton (1964) published a tentative test of efficacy of 5 commercially-available dog repellents. Papendeck (1968) conducted laboratory research on capsaicin by spraying the test dogs' muzzles and eyes. The agent produced an aversive response in dogs in the laboratory. Histological examinations showed that the spray did not cause lasting damage to the eyes.

The Environmental Protection Agency has developed a method that has been used as an efficacy test for aerosol dog repellents. Refer to proposed efficacy test TSD #1.504 (Exhibit 21).

1. Exhibit 21.

The method listed below is published by the Agency in its "Manual of Biological Testing Methods for Pesticides and Devices, Volume 1." A copy may be obtained by writing to the Product Manager #16, Insecticide-Rodenticide Branch (TS-767), Environmental Protection Agency, Washington, D.C. 20460. Users are advised that this BFSD (Benefits and Field Studies Division) method is subject to review and possible revision by the Agency and by the ASTM Committee E-35 on Pesticides.

Exhibit 21 (TSD # 1.504) is a proposed efficacy test for aerosol dog repellents that are designed to reduce damage to plastic garbage bags.

2. Reproductive inhibitors.

Balser (1964) specified the attributes of an ideal reproductive inhibitor. It must be effective in a single oral dose, offer a wide margin of safety between the effective dose (ED) and lethal dose (LD), remain chemically stable at an ED of less than 500 mg, be relatively tasteless and odorless, and have a sterility effect for one breeding season. The author's protocol was tried with the synthetic estrogen diethylstilbestrol.

Yasmuth et al. (1970) tested ovarian suppressants for dogs. They subcutaneously administered 2 ml medroxyprogesterone acetate to 60 free-roaming females. Blood tests, counts of mortality, and frequency of litters were taken. All females had at least 1 litter before treatment. Observation were made for a year.

Lunnen et al. (1974) published a laboratory protocol for inducing impotency in male dogs. Male beagles were immunized with a purified bovine lutenizing hormone. The dogs were sacrificed at the end of the experiment. The testes, epididymides and prostate, adrenal, thyroid, and anterior pituitary glands were excised and measured for weight differences.

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F. Browsing animal repellents.

Animal repellents are now widely used in the United States. In general, two types of repellents have been used for deer: odor repellents and taste repellents.

There are several methods for testing the efficacy of repellents on vegetation in relation to deer damage. The oldest method has been to lay out line transects and establish plots in treated and untreated areas (Besser and Welch, 1959). The most widely used method (Duncan and Whitaker, 1959), however, is to survey transects in the field on treated and untreated areas. These methods require a long period of time and the results are difficult to evaluate.

Another method involves pen tests where materials are treated and then presented to the test animals. An additional modification consists of providing pens with captive animals and planted blocks of treated and untreated seedlings. These pen tests yield more controlled data. In these experiments, the seedlings are planted in 2-1/2- and 1-acre (1 and 0.4 ha) enclosures holding deer and hares. A control and a repellent planting are used to evaluate the test chemical. Dodge, Loveless, and Kverno (1967) modified the techniques for the planted enclosures. These tests were randomized complete block design in which 10 blocks with 10 trees per block for each of the treatments were used. The untreated control pen must be observed routinely until 60 to 80% of the reference seedlings sustain animal damage.

Campbell and Bullard (1972) devised a preference test design using an apparatus which presents 2 food samples. The arrangement can be quickly changed to offer 2 more small dishes of a test material. These presentations are made so that at least 2 completely different sets of experiments can be executed at one time. Four to 10 deer are routinely tested once or twice daily for 1 or 2 weeks. The test consists of at least 10 to as high as 30 choices. Normally a deer can complete 20 choices in about 6 minutes. The animals are tested against a repellent standard. One objective of the test is to obtain marginal acceptance.

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VII. Field census techniques.

Population censusing under field situations is essential to adequately evaluate many vertebrate pest control agents. Some means must be used in determining the number of animals before treatment and again at post-treatment to establish efficacy. Censusing techniques are fundamental to all population studies designed to measure the influence of chemicals on the target or non-target populations.

Censusing techniques for the orders Rodentia and Lagomorpha are numerous and varied. Probably no technique is perfect; each has its strengths and weaknesses. The researcher must make every effort not only to determine which methods of population estimation are best suited to his purposes but also to apprise himself of all the weaknesses of his chosen methods. This enables the best interpretation of the results within the framework of his outlined test methods or procedures. In a general vein, Murray (1957) warns of the danger of error in most censusing techniques due to the selective distribution of animals according to habitat type within an area being censused. Censusing methods which in themselves affect the population (for example, fatal trapping, shooting, etc.) should not be used unless statistical procedures can be applied to correct for these effects. When practical, more than one censusing procedure should be used.

Many comparisons of techniques are available. Edwards and Eberhardt (1963) tested various methods of estimating a population of cottontail rabbits (*Sylvilagus floridanus*) from live-trapping data against a known population. They suggested, with some qualifications, that linear regression or maximum likelihood estimation (MLE) methods were the most useful. When several procedures were tried on grey squirrels (*Sciurus carolinensis*) in Maryland, Flyger (1959) found that a Schnabel (1938) population estimate, based on what Flyger termed "trap-sight records," was best for estimating the population. He used visual sightings because he felt that it lessened bias injected by trap-shy or trap-prone animals. The Schnabel method is a variation of the Lincoln Index that uses the average of a series of ratios rather than one, and is considered, therefore, to be less prone to sampling error. Nixon et al. (1967) also felt that population estimates of squirrels (*S. carolinensis* and *S. niger*) from the Schnabel method were low because of capture frequency variation for different individuals. They preferred the simplified MLE method (Edwards and Eberhardt, 1967).

Robinette et al. (1974) conducted tests of 10 different types of strip censuses. The 3 most satisfactory methods were those of Kelker (1945), Anderson and Pospahala (1970), and King. [King's method was described by Leopold (1933) in his book on game management.] Hayne (1949) presents a critical evaluation of the King method.

Beaver census techniques are reviewed and evaluated in light of an extensive census operation by Hay (1958). After checking several census methods, he found aerial counting of food caches to be the most practical and reliable.

Forest rodent censusing to determine conifer seed depredation potential provides an interesting example of a situation where the techniques used are not aimed toward absolute enumeration, but toward population indices for use in evaluating rodent control operations. Lines of live or snap traps are often used for these determinations. However, some researchers feel that the "seed-spot" census method is more useful to foresters than trapping because of its relative ease in use (Tevis, 1956; Moore, 1950). Tevis discusses the method thoroughly. It is based on the assumption that the percentage of piles of seeds placed in an area that are disturbed is proportional to the number of seed eaters in that area. Moore gives a key for determining what species disturbed each seed spot.

Emlen et al. (1949) reviewed Norway rat (*Rattus norvegicus*) census methods in some detail and discussed the shortcomings of some of the various methods. Chitty (1942) and Bentley et al. (1957) used wheat consumption as a population index for Norway rats in sewers. Rohe (1966) gives guidelines for censusing of roof rats (*Rattus rattus*); he used snap-traps and nontoxic paraffin-grain bait blocks to test for the presence of rats and their relative numbers. Nelson and Clark (1973) published a method for correcting trapping results by taking into account sprung traps in calculating catch effort. Emlen et al. (1957) and Tester and Emlen (1960) tested dropping boards as a method of obtaining population indices.

Census methods for pocket gophers (*Geomys bursarius*) and moles (*Talpa europa*) are presented by Beck and Hansen (1966) and Mead-Briggs and Woods (1973), respectively. Estimating pocket gophers by counting new signs was also reported by Reid et al. (1966).

When faced with the task of censusing a population, 2 sources for background information are Dice (1941) and Davis (1956). Dice reviews many of the methods for dealing with mammalian populations. Davis gives an outline of field and laboratory methods for accumulating and analyzing data on rodent populations. In addition to these sources, Chitty and Shorten (1946) present a compilation on the techniques for the study of Norway rats. And, finally, Tepper (1967) compiled a bibliography entitled, "Statistical Methods in Using Mark-Recapture Data for Population Estimations."

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